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Journal of Milk and Food Technology

NATIONAL MASTITIS COUNCIL ANNUAL MEETING

FEBRUARY 4-6, 1974

Shearton-Jefferson Hotel,

St. Louis, Mo. 63101

(See Program Highlights — Page 1)

Official **Publication**

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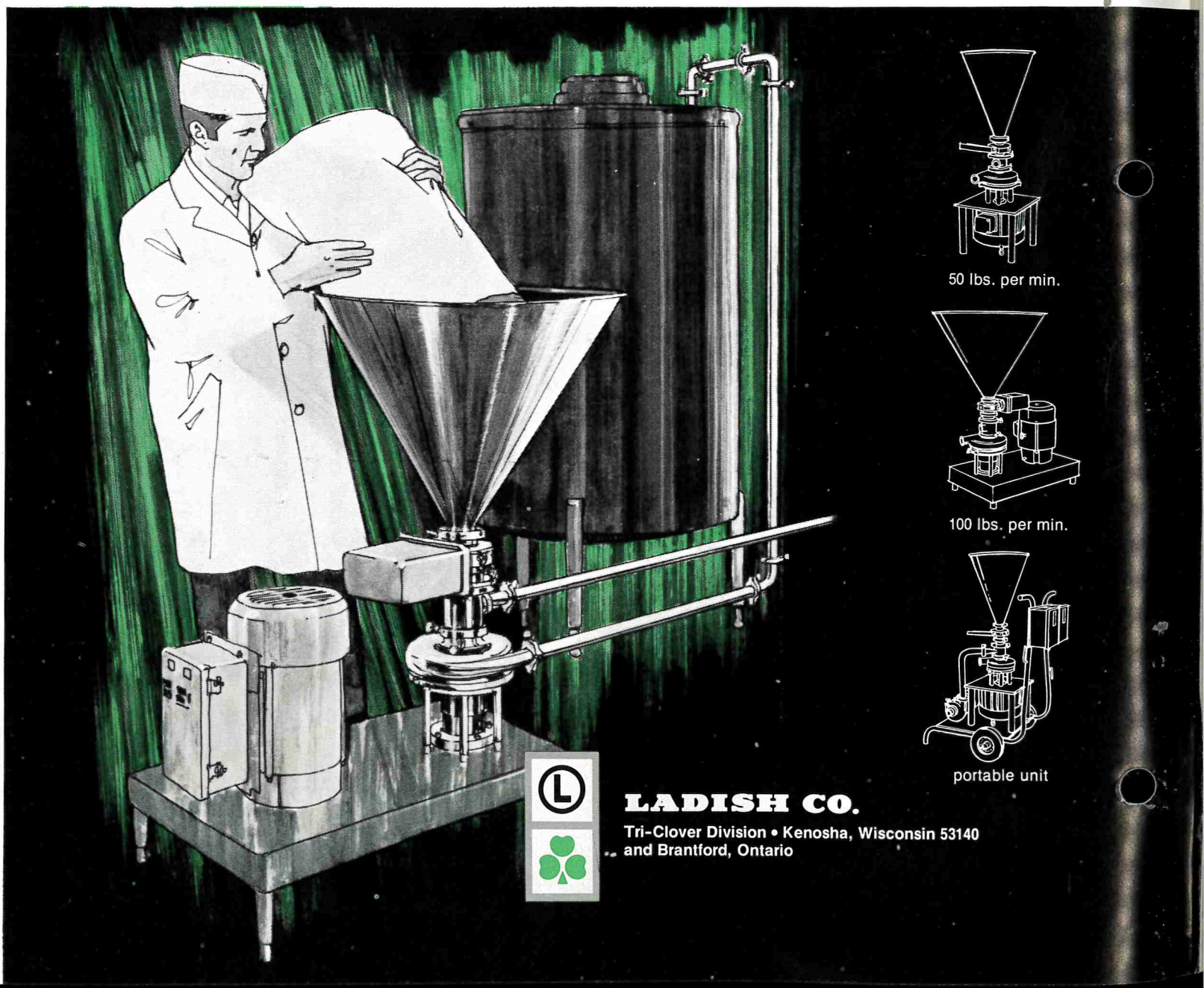

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NATIONAL MASTITIS COUNCIL ANNUAL MEETING

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February 5-6, 1974

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the 13th Annual Meeting of the National Mastitis Council.

A feature of the meeting will be the introduction of new NMC educational publications and slide sets:

(1) Money Returns from an Effective Program of Mastitis Control

(2) Procedures for Handling the Mastitis Problem Herd

(3) Mastitis Treatment Guidelines for Dairymen

(4) NMC Rules for Good Milking Technique

Further program highlights: An international authority, Dr. C. C. Thiel of the National Institute for Research in Dairying, Reading, England, will address the meeting on two subjects: (1) Recent Information on Mechanics of Teat Cup Action, and (2) How Vacuum Fluctuations Cause Mechanical Transport of Bacteria into the Teat.

Two presentations will deal with the import of milking system design: Mr. Francis F. Smith, Los Angeles County Dairy Farm Advisor, will present the rationale for design; Dr. John S. McDonald, National Animal Disease Laboratory, will discuss the effect of design and function on udder health.

Mr. Edward A. Fiez, University of Idaho, will review the application of milking principles to quality milk production.

Dr. Roger P. Natzke, Cornell University, will present papers on (1) Relationship of Bulk Milk Somatic Cell Levels and Mastitis Incidence, and (2) Long Term Effect of a Teat Dip/Dry Cow Treatment Program.

An update on the status of coliform mastitis will be given by Dr. R. B. Bushnell, University of California, Davis.

Reflections of a Dairy Farmer on the NMC: Past, Present, and Future will be presented by Arlen Schwinke of Morrison, Missouri.

Dr. R. D. Mochrie, North Carolina State University, will moderate a panel discussion on factors affecting milking performance. Panel participants include: Messrs. Edward A. Fiez, John S. McDonald, Francis F. Smith, and C. C. Thiel.

Make your plans to attend this meeting. It will start at 8:45 a.m. on February 5 and will adjourn at noon on February 6. Request advance registration form from National Mastitis Council, 910 - 17th Street, NW, Washington, D. C. 20006.

Send request for room reservation directly to the Sheraton-Jefferson Hotel, St. Louis, Mo. 63101. Ask for special NMC rates: Single — $15.50 per day; Twin — $21.50 per day.

Harvey J. Wilhelm, President
National Mastitis Council

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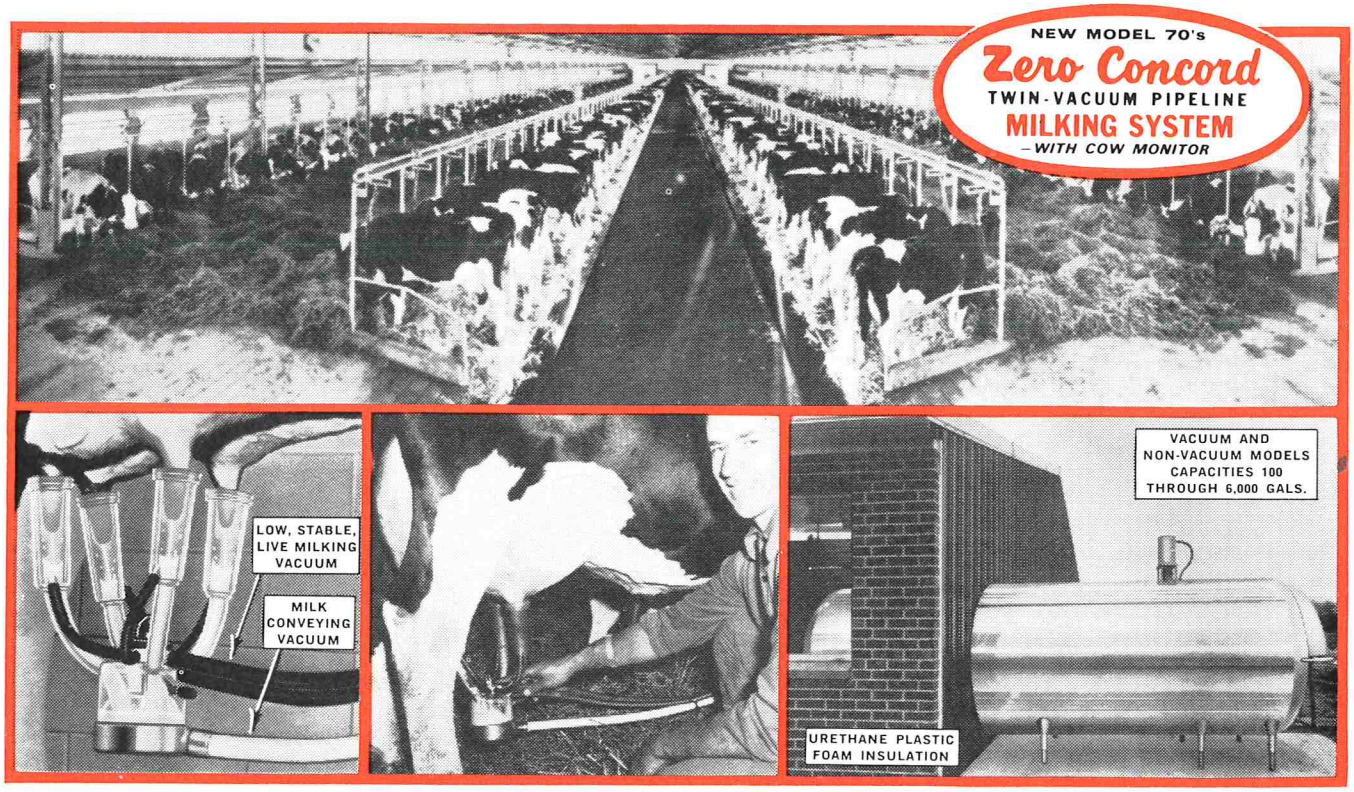

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COMPILED AND EDITED BY

J. C. FLAKE, A. E. PARKER, J. B. SMATHERS, A. K. SAUNDERS AND E. H. MARTH

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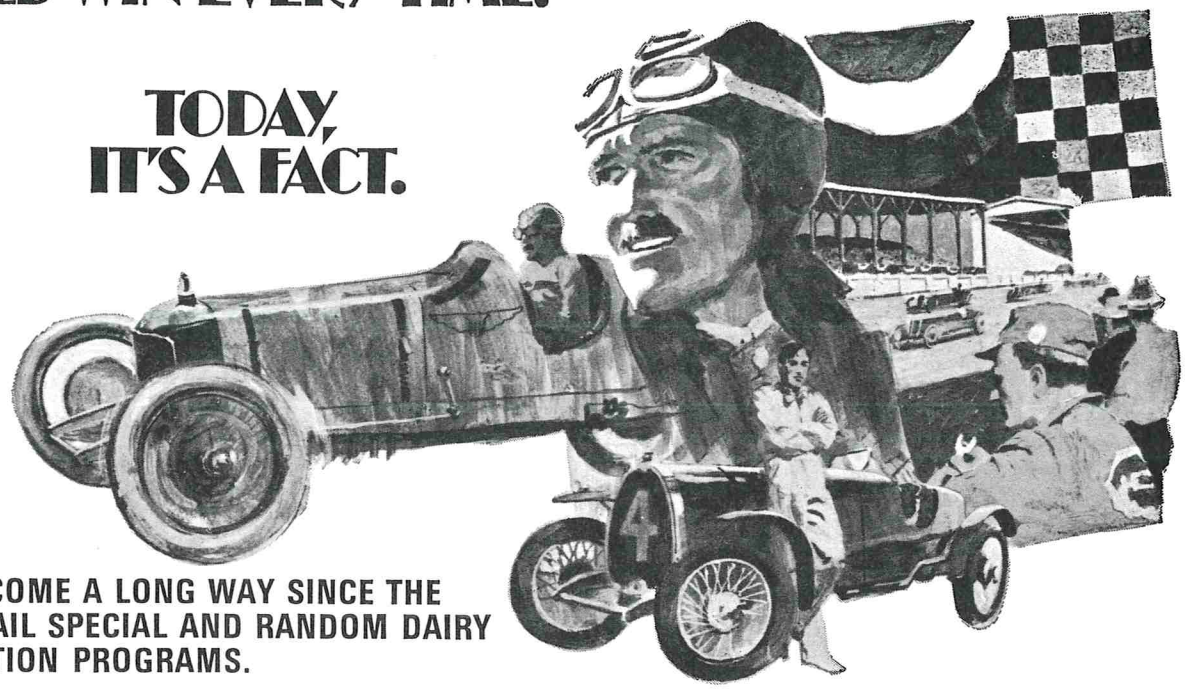

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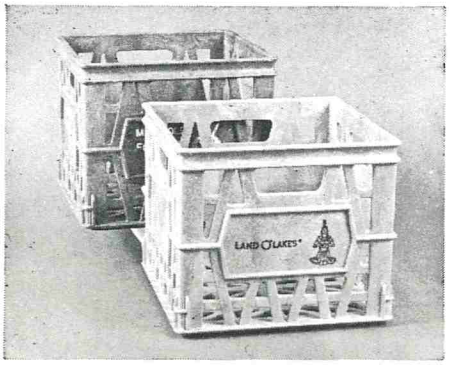

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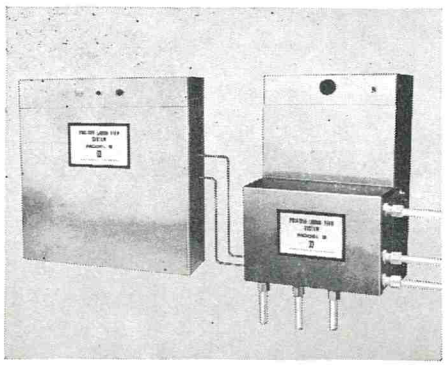

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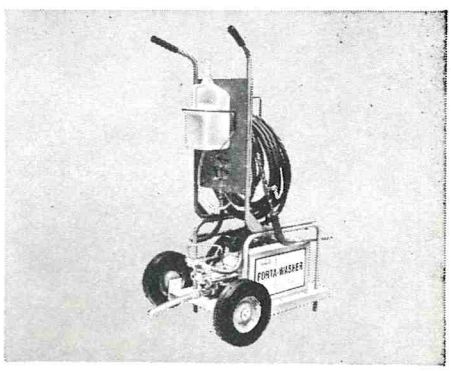

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FLAVOR DEVELOPMENT AND MICROBIOLOGY OF SWISS CHEESE–A REVIEW

III. RIPENING AND FLAVOR PRODUCTION[1, 2]

T. Langsrud and G. W. Reinbold
Department of Food Technology
Iowa State University, Ames, Iowa 50010

(Received for publication January 31, 1973)

Abstract

This paper, the third of four, discusses the ripening and flavor production processes of Swiss cheese. First, general ripening changes are reviewed; then, a discussion of eye formation and body and texture changes follows. The important flavor compounds found in Swiss cheese are examined in detail beginning with consideration of the analytical methods used to isolate and measure fatty acids. The different volatile and nonvolatile fatty acids and their importance in Swiss cheese are considered, as well as production of propionic and acetic acids from lactate, production of keto acids, and lipolysis. Then, analyses for and significance of carbonyl compounds, alcohols, esters, lactones, hydrocarbons, and diacetyl are presented. Possibilities for production of these compounds by microorganisms are given. Subsequent sections deal with nitrogeneous compounds, degradation of caseins to peptides and amino acids and their importance to flavor, and end with a review of sulfur compounds present.

After Swiss cheese has been removed from the press, brined, and prestored for 7 to 14 days, it is transferred to the hot room where the temperature is 21 to 25 C (70 to 77 F). During the period in the hot room cheese becomes sufficiently elastic and the principal ripening of the cheese, with active growth of propionibacteria, takes place, forming the typical eyes of Swiss cheese *(430)*. When the eyes are large enough, the cheese is moved to the curing cellar or "finished cooler" and kept there at 2 to 5 C (35.6 to 41 F) for 2 to 9 months. Ripening for at least 6 months should develop the fine, full flavor characteristic of a well-ripened Swiss cheese *(116)*; however, most commercial Swiss cheese sold in the United States today is much younger when sold *(345)*.

General Aspects of Swiss Cheese Ripening

During the time in the press, high-temperature lactic-acid bacteria rapidly hydrolyze lactose in the young cheese to glucose and galactose *(338)*, which, by the Embden-Meyerhof pathway, is oxidized to lactic acid by the same bacteria *(8)*. Production of lactic acid in the cheese produces a readily assimilable substrate for the propionibacteria. Propionibacteria were first found by Sherman *(393)* to be essential for eye formation as well as production of the characteristic sweet flavor of Swiss cheese.

A large part of the lactic acid in Swiss cheese is produced by homofermentative lactic-acid fermentation, but a small portion also is produced through the heterofermentative pathway. Moderate amounts of acetic acid are found in the cheese after pressing *(218)*. Because of this heterofermentative activity, carbon dioxide, which induces growth of propionibacteria, also has been found *(150)*. Lactic-acid bacteria also reduce the redox potential of the Swiss cheese to −200 mv, which is favorable for growth of propionibacteria *(199)*. Another important factor for growth induction of propionibacteria is the pH-value of the cheese out of the press. The pH must be between 5.0 and 5.3 to produce good Swiss cheese. If the pH is lower than 5.0, growth of propionibacteria will not take place *(431)*.

During the hot room and curing room treatment of Swiss cheese, the number of *S. thermophilus* and *L. helveticus* decline *(79)*, even though it has been stated that mixed cultures of *S. thermophilus* and *L. helveticus* may grow at temperatures as low as 15 C (59 F) *(171)*. The primary reason for this numerical decline is the elimination of lactose as an energy source and the formation of some metabolic products in the cheese that may have an adverse effect on growth, especially as the cheeses are ripened near their minimum growth temperature. *Lactobacillus casei*, however, grows during ripening, and some investigators consider it an important ripening organism *(79)*.

Growth of propionibacteria is induced in the hot room. Frazier and Wing *(121)*, in 1931, stated that significant growth took place after about 3 weeks in the hot room, when eye-formation begins. After 2 weeks in the hot room, the population of propionibacteria had increased 3 to 980 times the corresponding numbers present in curd in the press *(236)*. This

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[1]Journal Paper No. J-7505 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1839.
[2]This review is appearing in four parts: I. Milk Quality and Treatments and II. Starters, Manufacturing Processes and Procedures appeared in earlier issues; IV. Defects will appear in a subsequent issue. Literature citations will follow part IV.

number was dependent on the initial pH of the cheese out of the press. Maximum numbers of propionibacteria were found after 6 or 8 weeks when the initial pH had been between 5.15 and 5.37. With initially lower pH values, maximum population occurred between 10 and 13 weeks. The population of propionibacteria showed a positive correlation with pH from the press. After the maximum number of propionibacteria was reached and sufficient eye formation had occurred, the cheese was transferred to the cold room. The number of propionibacteria slowly decreased during further ripening in the cold room *(79, 236)*, even though some strains of *Propionibacterium* can grow at temperatures as low as 7 C (44.6 F) *(319)*. The distribution of propionibacteria in conventional Swiss cheese with rinds has been determined *(347)*. Very few propionibacteria (1×10^6/g cheese) were found just below the surface of the cheese. Samples at 2.22 cm (7/8 inch) below the surface contained many more propionibacteria (74 to 1,200 $\times$ 10^6/g cheese) than the exterior portion, and the number progressively increased toward the center of the cheese (410 to 2,200 $\times$ 10^6/g cheese). Decrease of propionibacteria toward the exterior possibly results from such unfavorable conditions as lower moisture, higher salt concentrations, and higher redox-potential. Rindless Swiss cheese has a more uniform distribution of propionibacteria because of the alleviating influence of the impermeable wrapper.

During curing, diverse chemical changes are brought about in Swiss cheese because of glycolysis, lipolysis, and proteolysis. Lactic acid is degraded to propionic and acetic acid, and protein hydrolysis takes place. Flavor development depends on the relative amounts of lower fatty acids produced *(245)*. Lower free fatty acids are related to the lactate content of the Swiss cheese, and lactate content is related to the lactose content in the cheese the day after manufacture. Cheese of good quality contains large amounts of propionic and acetic acid and small amounts of butyric and higher fatty acids. With higher amounts of butyric and higher fatty acids, Swiss cheese flavor is strong and rancid *(245)*. Sahli and Lehmann *(370)* showed that a well-ripened Swiss cheese contained 0.3% (by weight) acetic acid and 0.4% propionic-acid but, also at least 0.012% butyric acid.

Proteolysis takes place during curing and the content of free amino acids in Swiss cheese increases. Free amino acids in amounts from 3.2 to 4.1 mg% were recognized as normal for Swiss cheese *(143)*. Protein breakdown is delayed in Swiss cheese containing copper *(190)*. In a study at Iowa State University on Iowa-style Swiss cheese, however, no retardation of proteolysis was observed, and, in most instances, higher proteolytic values were observed (Maurer and Reinbold, unpublished results). Propionibacteria do not affect proteolysis significantly *(207)*. Most of the characteristic sweet flavor of Swiss cheese has been attributed by some workers *(449)* to the proline content, which is higher in Swiss cheese than in any other cheese. Other workers *(226)* believe that proline and the ratio of propionic acid to proline is important for Swiss cheese flavor. Besides these two important groups of compounds, a large number of volatile carbonyl compounds in low concentrations has been isolated, and these carbonyl compounds may be important for flavor *(244)*.

The pH of the Swiss cheese rises in the cold room, the hot room, and the curing room. Cheese out of the press has a pH from 5.1 to 5.3, and these values rise about 0.05 to 0.1 pH unit in the cold room *(116)*. The increase in pH in the hot room is much faster and the pH will be about 5.5 at the time the cheese is moved to the curing room *(116)*. The pH after 2 to 3 months should not be less than 5.5 *(447)*. If this pH is not reached, normal ripening does not occur. Sahli and Lehmann *(370)* reported that high-quality Swiss cheese has a pH value between 5.64 and 5.70.

The fat content in dry matter of a high-quality Swiss cheese should be about 48% *(370)*. The distribution of fat in Swiss cheese was studied by Oehen and Ryser *(299)* who found that fat content varied if the cheese was sampled on the outside or in the middle of the wheel. Moisture content also varied with the sampling site in the cheese *(299)*. Sahli and Lehmann *(370)* found average moisture in high-quality Swiss cheese to be about 36.9% at 3 months and 34.5% after 6 months. Rindless block Swiss cheese will contain perhaps 2.0% more moisture *(345)*. Green cheese containing more than 39.7% moisture results in poor quality cheese *(371)*. This detrimental effect is most evident in relationship to eye formation, and the defect known as "oversetting," too numerous and small eyes, will be very pronounced *(372)*. High moisture content may be the result of conditions in the manufacturing process such as low-fat milk, soft-curd milk, slow acid-development, low setting temperature, fine harping, low cooking temperature, and shortening of each step in the procedure *(371, 372)*.

A high-quality Swiss cheese should contain eyes of proper size and form, and the eyes should be evenly distributed in the cheese. Normal eyes should have a diameter from 1.25 to 2.54 cm (0.5 to 1 inch) and should have 2.54 to 7.62 cm (1 to 3 inches) between them *(141)*. Gas in the eyes of a normal cheese consists of carbon dioxide and nitrogen. Nitrogen is occluded from the air *(60)* and carbon dioxide is produced by propionibacteria. Because of the rela-

tively few eyes produced in Swiss cheese, it is improbable that eyes are formed at the location of colonies *(60)*. Gas, as it is produced, may diffuse through the curd and collect at weak points in the cheese where the eyes will be formed *(60)*. The ripening of Edam cheese has been followed with X-rays *(385)*. Eye formation could be followed but this technique was difficult to apply to large blocks of Swiss cheese. With smaller blocks of Swiss cheese, van Allemeersch and Devogelaere *(4)* established, by X-ray analysis, that the eyes of Swiss cheese are usually visible after ripening at 13 C (55.4 F) for 12 days. The constant growth of the eyes can be clearly followed to the end of the storage period in the hot room at 20 C (68 F). A marked similarity was observed in all instances between the X-ray image and a corresponding section of cheese. Presence of numerous small openings detected by X-ray analysis in young cheese was most often associated with the texture defect "oversetting" (Vielsetzer) *(4)*. Copper-containing cheeses were shown to have fewer and larger eyes than control cheeses, and, at higher copper concentrations (11 mg/kg), they required a longer ripening time for eye development. Velocity of carbon dioxide production was markedly reduced (Maurer and Reinbold, unpublished results).

Relative humidity in the ripening room also has an influence on eye formation. Uneven relative humidity caused uneven eye formation in Swiss cheese because of differences in rind resistance of the cheese *(424)*. This observation, of course, would not apply to rindless block Swss cheese cured in an impermeable wrapper *(345)*. If cheese held at regular curing temperature showed evidence of too many or too large eyes, further swelling could be avoided by placing the cheese in a cold room at about 4 C (39.2 F) *(18)*. The swelling effect could result from continued carbon dioxide production from propionibacteria in Swiss cheese at low temperatures. Park et al. *(319)* showed that strains of propionibacteria may grow at temperatures as low as 7.2 C (45 F).

During ripening of Swiss cheese, the body gradually becomes softer, less tough, and less elastic. These changes probably reflect the solubilizing properties of proteolytic enzymes *(116)*. Watson *(456)*, however, observed that production of a desirable soft texture in the cheese was not necessarily coincident with the highest degree of proteolysis, quite the opposite actually being true. Variation in the colloidal structure of cheese, as shown by water binding, must be important in body characteristics. Swiss cheese tends to become more firm and less pliable as it loses moisture *(209)*.

Body characteristics of Swiss cheese are due to the nature and state of the colloidal nitrogenous substances *(210)*. Elasticity of cheese is highly temperature variable. Texture varies, not only from cheese to cheese, but also within an individual cheese *(211)*. Body characteristics of Swiss cheese were attributed by Koestler *(212)* to "hard nitrogenous substances" resulting from high cooking temperature, which gives the cheese, together with a relatively low moisture content, its characteristic properties. Cheese physical properties are determined not only by chemical transformations occurring during lactic and propionic-acid fermentation, but also by the inner structural relation of the cheese curd. Characteristic for Swiss cheese is a high degree of elasticity, moderate firmness of the body, and high water-binding capacity. Mocquot et al. *(272)* found that total and plastic deformation of Swiss cheese was highly correlated with temperature and moisture. Eye number and size increased as total and plastic deformation increased. Rheological studies of Iowa-style Swiss cheese containing different amounts of copper showed that copper content did not influence strength of the cheese but that an increase in copper content was highly correlated with an increase in elasticity and viscosity (Maurer and Reinbold, unpublished results).

Changes in Swiss cheese curd, observed using electron microscopy *(366)*, seemed to support the belief that serum κ-casein forms the initial linking element between casein particles. Further changes in the cheese curd during syneresis, pressing, salting, and ripening were accompanied by a progressive increase in homogeneity of the casein matrix and in deformation of fat globules. Bacteria were found mainly in capillaries and nodes that were probably filled with whey, surrounded by zones of protein breakdown, containing crystal aggregates, believed to be of the less soluble amino acids. For example, Flückiger and Schilt *(113)* found tyrosine produces white crystals in Swiss cheese.

FLAVOR PRODUCTION IN SWISS CHEESE

Ripening of Swiss cheese is a slow process. Controlled, accelerated ripening would be of great help for flavor studies of Swiss cheese. Kristoffersen et al. *(225)*, in 1967, developed a procedure for accelerated ripening of Cheddar cheese that involved use of a liquid cheese product, which acquired an intense, sharp, balanced Cheddar flavor in their judgment. Such factors as storage temperature, salt concentration, agitation, mineral-vitamin addition, and storage of frozen curd also were investigated *(399)*. Singh and Kristoffersen later applied this method to Swiss cheese curd *(398)* and found that development of characteristic flavor was accompanied by increased

pH, decreased lactic acid, and formation of active sulfhydryl groups *(400)*. Head-space volatile compounds produced were quantitatively similar to those of aged Swiss cheese. Development of Swiss cheese flavor in the slurry was enhanced by reduced glutathione, was directly related to initial pH and amount of propionic-acid bacteria, and was inversely related to salt concentration. Swiss cheese curd manufactured only with *S. thermophilus* and propionibacteria, instead of *L. helveticus, S. thermophilus,* and propionibacteria adversely affected the flavor of the Swiss cheese slurry *(400)*.

"Component balance theory"

Earlier belief was that only one or a few compounds caused Swiss cheese flavor. Propionic and acetic acid developed parallel to flavor development, and Babel and Hammer *(18)* proposed that propionic acid was the most important contributor to Swiss cheese flavor. Later, Virtanen and Kreula *(449)* attributed the characteristic sweet flavor of Swiss cheese to proline content. Hintz et al. *(165)* found that a minimum proline content of 2.0 mg/g and a minimum propionic acid content of 5.0 mg/g of cheese were necessary to produce a typical Swiss cheese flavor.

This concept has now generally changed and has been replaced by the "component balance theory" of Kosikowski and Mocquot *(215)*, which states that a relatively small number of compounds are responsible for the differences in flavor of different cheese varieties. The more important components of cheese flavor include certain fatty acids *(321)*, aldehydes *(188)*, methyl ketones *(152)*, diacetyl *(52)*, amines *(397)*, peptides *(153)*, sulfur compounds *(453)*, and amino acids *(449)*; other compounds, such as esters *(69)*, alcohols *(125)*, partial glycerides *(265)*, lactic acid *(281)*, and salt probably also have some effect. Neutral fat, para-casein, and moisture cannot be ignored since taste threshold concentrations of flavor compounds vary in fat and water solutions *(321)*. These compounds are found in all types of cheeses to give them a basic cheese flavor, but it is the amount and proportion of a few typical compounds that produces the typical flavor of a certain cheese variety. Extreme shifts in proportions will lead to abnormal flavors. All components found in Swiss cheese may be considered important because of synergistic effects, even if they are found in lesser amounts than their threshold value. Other compounds present in amounts that are detected as off-flavors may still be essential for typical flavor when present in lower amounts *(115)*.

Acids in cheese

Volatile fatty acids, fatty acids, and keto acids are always found in Swiss cheese. Volatile fatty acids probably are most important from the aspect of Swiss cheese flavor, but other acids may be important for their contribution to background flavor. In this part of the review, production and flavor of acids is discussed.

Analytical methods. With development of chromatographic methods, the number of reports concerning the presence of various acids in cheese has increased. In older literature, only reports about volatile fatty acids recovered through steam distillation are found *(18, 310)*. Paper chromatography was first used for identification of volatile acids *(166, 256)*, but Lindqvist et al. *(256)* found only propionic and acetic acid by the method they used. Harper *(146)*, in 1953, developed a column chromatographic method with silicic acid as filling material for quantitative determination of acetic, propionic, and butyric acid in cheese. Samples were prepared by adding sulfuric acid to 5 g cheese to bring the pH to 1.7 to 2.0, and the samples were added directly to the column. Besides the volatile acids, pyruvic acid also was separated. Thin-layer chromatography also has been used for analysis of volatile fatty acids from Swiss cheese *(16)*.

Gas chromatographic methods in particular were shown to be useful in analysis of volatile and non-volatile fatty acids *(36, 176, 291, 321, 421)*. Volatile acids could usually be separated from cheese by steam distillation of an acid cheese-slurry *(321, 421)*, or an acid water-filtrate could be used *(16)*. To determine higher fatty acids, Bills and Day *(36)* made a thick, acid, cheese slurry that was packed in a centrifuge tube and heated to 40 C (104 F), then, it was centrifuged and the fat layer used for analysis. Fatty acids were methylated before gas chromatography was done. This method does not permit total recovery of fatty acids, but is most commonly used. Volatile and non-volatile fatty acids also can be isolated by extraction with organic solvents *(239)*. A glass column seems preferable to a steel column for analysis for free volatile fatty acids *(291, 314, 315)*.

Volatile and nonvolatile fatty acids in Swiss cheese. In 1904, Orla-Jensen *(310)* found that Swiss cheese contained propionic and acetic acid in the mol proportion 2.3 to 1. It was not until 1921, however, that Sherman *(393)* concluded that propionibacteria were necessary to ensure proper eye and flavor formation. As had been noted many times already, these bacteria produce propionic acid, acetic acid, and carbon dioxide from lactose and lactate *(392)*.

Swiss cheese with a conspicuously sweet flavor was found by Babel and Hammer *(17)* to contain a higher volatile acid content than cheese lacking a definite sweet flavor. Addition of propionates to processed Swiss-type cheese lacking in flavor produc-

TABLE 1. VOLATILE AND NONVOLATILE FATTY ACIDS IN SWISS CHEESE COMPARED WITH CHEDDAR, TILSIT, AND EDAM[a]

Acids	Fatty acid content in cheese											
				Swiss[e]			Emmental[e]		Emmental[f] tear fluid[g]	Cheddar[h]	Tilsit[c]	Edam[c]
	Swiss[b]	Emmental[c]	Emmental[d]	A	B	C	D	E				
	(mg)											
Formic	–	9.2	–	31.4	–	–	–	–	6.9	–	4.6	–
Acetic	260	252	232	356.8	183.5	253.9	372.4	368.0	51.6	83.1	105.6	144.0
Propionic	560	414.4	470	218.1	386.8	289.9	591.9	494.1	69.6	–	14.8	11.8
n-Butyric	58	0.88	7.8	8.6	33.7	12.7	32.9	8.9	1.8	12.7	3.5	2.6
2-Methyl butyric	–	–	–	3.2	2.9	1.2	10.0	2.2	–	–	–	–
3-Methyl butyric	–	–	–	4.7	–	–	1.3	–	3.5	–	–	–
Caproic	–	–	–	3.7	22.5	7.0	11.5	4.1	3.8	3.3	+	+
Caprylic	–	12.68	–	3.8	21.6	7.9	9.4	4.0	5.2	4.3	7.2	7.2
Capric	–	28.71	–	8.6	19.0	9.8	11.3	4.0	–	5.3	26.1	29.5
Lauric	–	45.0	–	13.3	31.0	15.7	17.4	7.4	–	8.3	31.0	40.0
Myristic	–	–	–	31.8	96.6	52.3	59.2	25.8	–	23.6	–	–
Palmitic	–	–	–	58.7	309.1	139.0	172.7	72.1	–	51.0	–	–
Stearic	–	–	–	9.7	65.0	33.4	62.9	19.3	–	19.2	–	–
Oleic	–	–	–	36.3	202.6	98.5	178.7	48.1	–	45.8	–	–
Linoleic	–	–	–	6.5	27.2	14.3	23.8	4.7	–	8.1	–	–
Linolenic	–	–	–	7.8	23.1	11.5	17.5	6.2	–	4.3	–	–
Higher fatty acids	340	–	–	–	–	–	–	–	–	–	–	–

[a]Analysis values per 100 g cheese.
[b]Hintz et al. *(165)*.
[c]Schormüller and Langner *(378)*.
[d]Kiermeier et al. *(195)*.
[e]Langler and Day *(243)*.
[f]Schormüller et al. *(379)*.
[g]Analysis values per 10 g tear fluid.
[h]Bills and Day *(36)*.

ed a sweet flavor. This suggests that added propionates have a positive effect on Swiss cheese flavor.

High-quality Swiss cheese contains relatively large amounts of acetic and propionic acid with little or no butyric acid and a small portion of higher fatty acids *(217)*. Krett and Stine *(217)* observed that, in Swiss cheese with a flat flavor, the acetic-acid content was comparable to normal cheese but little or no propionic or butyric acid was present. Highly objectionable flavor was found in cheese containing large amounts of butyric acid. Krett et al. *(218)* later determined the amounts of volatile fatty acids in Swiss cheese. In cheese coming out of the hot room, the amounts of fatty acids of the cheese in mg/100 g cheese were 167 acetic, 344 propionic, 18 butyric, and 30 higher volatile fatty acids. In 140-day-old cheese, the content was 290 mg acetic, 630 mg propionic, 38 mg butyric, and 50 mg higher fatty acids. Using Harper's method *(146)*, Hintz et al. *(165)* found acetic, butyric, propionic, and fatty acids of C_6 and greater chain lengths in all cheeses, and valeric acid was observed in all cheeses except one. Typical values in an 11-month-old cheese of good quality were 3 mg acetic acid, 5 mg propionic acid, 1.1 mg butyric acid, 0.19 mg valeric acid, and 24.2 mg of the higher acids per gram of cheese. No correlation was found between free fatty acids and the age of the cheeses. Comparable values for these acids in Swiss cheese also were found by Kurtz et al. *(236)*. The propionic-to-acetic acid ratio in cheese was found to be 0.8 to 1.0, but this amount of acetic acid may be high because pyruvic acid was eluted with acetic acid *(236, 241)*. After assessing aqueous solutions of propionic acid and propionate, Kurtz et al. *(236)* stated that propionic acid did not seem to cause the sweet flavor in Swiss cheese, but that it still is an important factor in the flavor complex. This refutes the claim of Babel and Hammer *(17)*, that propionic acid is responsible for the sweet flavor of Swiss cheese.

Schormüller and Langner *(378)* found the fatty acids shown in Table 1 in Swiss cheese by paper chromatography; C_4 and C_6 monocarboxylic acids were found only in small amounts. Patton *(322)* observed that Swiss cheese was characterized by a high proportion of propionic acid; he also detected 2- and 3-methylbutyric acids among the volatile fatty acids. n-Valeric acid was not detected, but Hintz et al. *(165)* who found this acid used Harper's method *(146)*, which does not separate the branched C_5-acids from n-valeric acid. Schormüller et al. *(379)* also found high amounts of propionic and acetic acid in the "tear fluid" of Swiss cheese and other volatile fatty acids in lower amounts (Table 1). In a comparative study between Gruyère and Swiss cheese, the amounts of volatile acids seemed a little higher in Gruyère *(239)*.

Langler *(241)* and Langler and Day *(243)* made detailed studies of the amounts of the volatile and other major fatty acids in Swiss cheese. Results are in Table 1, where the values for the free fatty acid composition of Cheddar cheese are included for comparison *(36)*. High-quality Swiss cheeses, including two imported samples, were analyzed *(241, 243)*. 2-Methylbutyric acid was detected in all cheeses and might possibly be important for flavor. Cheeses of the lowest quality also contained the lowest amount of this acid. 3-Methylbutyric acid seemed not important for the flavor complex since it was found in only two of the cheeses. The ratio of propionic to acetic acid indicated no simple relationship to flavor. Acetic, butyric, caproic, caprylic, and capric acid existed in Swiss cheese *(241, 243)* in amounts higher than their flavor-threshold values in water, milk, and oil as determined by Patton *(323)* and may, therefore, be of importance in flavor. By comparing the proportions of the esterified fatty acids of milk fat with those of the higher free fatty acids of Swiss cheese, the results suggest that these acids arise from a nonspecific hydrolysis of milk fat *(241)*, which also is believed to apply to Cheddar cheese *(36)*.

Propionic-acid production in Swiss cheese. For a detailed review of propionic-acid metabolism, the reader is referred to a review paper by Hettinga and Reinbold *(157)*. In this review, propionic-acid formation will be discussed primarily in association with Swiss cheese.

It has been known since 1906 that propionic acid in Swiss cheese was formed by fermentation of lactic acid by propionibacteria *(123)*. These organisms also may produce propionic acid from lactose, glucose, succinate, glycerol, pyruvic acid, and peptone, but the proportion of propionic acid to acetic acid varies with substrate *(392, 445)*. It also is known that their metabolism advances through phosphate esters *(412)*. So, it has been established, in general, that propionibacteria metabolize glucose and lactose through the Embden-Meyerhof scheme *(21, 412)*. A reaction scheme for production of propionic and acetic acid from glucose and lactate, was proposed by Allen et al. *(5)*; (see also reference *157*.)

In Swiss cheese, propionic acid is usually formed from lactate produced by the high-temperature starter bacteria. Lactate promotes growth of propionibacteria in cheese, and El-Hagarawy et al. *(101)* found that addition of lactate to milk increased acid production. No relationship was found between maximum amount of propionibacteria and amount of propionic acid produced *(102)*. With rapid growth in a broth medium, the proportion of propionic acid to acetic acid was about 2:1 for the first 9 days and 1:1 thereafter *(100)*. By addition of propionate and acetate in a proportion of 2:1 to a slurry from Swiss cheese, growth of propionibacteria was inhibited *(14)*. For normal Swiss cheese, the amounts of these acids are variable and, in many instances, too low to hinder late fermentation (Nachgärung). In good, nonperishable cheeses, the proportion of propionate to acetate averages about 1.98, and in perishable cheeses about 1.26.

TABLE 2. PRODUCTION OF VOLATILE FATTY ACIDS (C_2-C_4) DURING RIPENING OF SWISS CHEESE *(195)*

Age of cheese in weeks	Amount of fatty acids in 100 g cheese		
	Acetic acid	Propionic acid	Butyric acid
	(mg)		
1	47.0	0	0
2	48.3	0	0
3	50.0	37.3	0
4	106.9	109.9	0
5	110.4	141.0	0
6	152.0	211.1	0
7	180.9	355.5	0
8	204.8	375.0	2.2
10	231.0	471.6	5.3
11	231.2	472.5	6.4
24	232.4	470.0	7.8

Addition of nitrite (0.001 to 0.2%) to cheese milk inhibits propionic-acid fermentation in Swiss cheese *(195)*. Addition of hydrogen peroxide (0.035 to 0.17%) to cheese milk, followed by catalase treatment lowers the production of propionic acid, but raises that of acetic acid and increases protein breakdown. Heating the milk [65 to 72 C (149 to 161.6 F) for 40 sec] inhibits propionic-acid fermentation in the cheese to a degree dependent on the severity of the heat treatment.

High-temperature lactic-acid starters produce some acetic acid *(195)* in the cheese before propionibacterial growth is induced, so, before the cheese is moved to the hot room, acetic acid is found in higher proportions in the cheese than propionic acid. Table 2 shows the production of acetic, propionic, and butyric acid in Swiss cheese during aging *(195)*. By using *S. thermophilus* and *L. helveticus* as starter instead of just *L. helveticus*, the ratio of propionic to acetic acid was lowered *(199)*. Decrease in lactic acid in cheese milk promotes the propionic-acid fermentation because the pH of the cheese is raised, showing that pH regulates production of propionic acid by propionibacteria. Dilution of lactose by addition of water during cheese production raises the ratio of propionic to acetic acid *(199)*. Lactic-acid fermentation lowers the redox potential of the Swiss cheese to −200 mv, and propionibacteria raise it to −50 to −100 mv during growth. Addition of metallic iron and its chlorides promotes the propionic-acid fermen-

tation, even if it raises the redox potential *(199, 200)*. The proportion of propionic to acetic acid is strongly influenced by the presence of ionic iron. Ferrous hydroxide addition produces propionic acid as the primary end product but ferric hydroxide produces mainly acetic acid *(189)*.

The velocity of the propionic-acid fermentation depends on the bacterial producer of the lactic acid *(201)*. *Streptoccocus thermophilus* and *L. bulgaricus* showed no distinct differences, whereas the propionic-acid fermentation following growth of *L. lactis* is markedly slower. This depended on the lactic acid produced; as *S. thermophilus* produces the L (+) form, *L. bulgaricus* the L (+) D (−) form, and *L. lactis* the D (−) form of lactic acid.

Swiss cheese was made with either *S. thermophilus*, *L. helveticus*, or *L. lactis* as the high-temperature starter *(407)*. Time of lactic-acid formation during pressing of the curd had a definite influence on the total amount of lactic acid formed in the cheese. *Streptococcus thermophilus* produced lactic acid during the first hours of pressing, while *L. lactis* was late and slow in acid production. Mainly L(+) lactic acid was found in the cheese made solely with *S. thermophilus* and this optical form of lactic acid was then utilized very rapidly. In cheese with *L. helveticus*, both L (+) and D (−) lactic acid were formed in about equal amounts, but the L (+) lactic acid decreased the fastest. With *L. lactis*, the amount of D (−) lactic acid was highest and it decreased slower during the hot room period *(407)*. Cheese made with *S. thermophilus* and *L. lactis* alone were of inferior quality. This seems to support Kiuru's observation on the relationship between optical form of lactic acid produced and the velocity of the propionic-acid fermentation *(201)*.

Copper was found to have an inhibitory effect on propionibacteria (*280* and Maurer and Reinbold, unpublished results). With about 17 mg copper/kg of dry cheese the formation of propionic acid was severely retarded *(197, 198, 470)*. The lactic-acid content increased, and the content of volatile acids decreased, with increased copper levels in Swiss cheese (Maurer and Reinbold, unpublished results).

In an experiment with Swiss cheese produced in copper and steel vats, Steffen and Blanc *(407)* found that the total amount of lactic acid in cheeses from copper vats was lower than in cheeses from steel vats. This indicates that copper probably inhibits lactic-acid production by high-temperature lactic-acid bacteria *(407)*. Using an enzymatic method, Steffen *(406)* measured the amount of L (+) and D (−) lactic acid produced. Both L (+) and D (−) lactic acid were found in lower amounts in cheese from the copper vats than in cheese from steel vats. In both kinds of cheeses, the amount of L (+) lactic acid was highest. In the cheeses made in a copper vat, the lactic-acid content started to decrease after 30 days, while in cheese from a steel vat, the L (+) lactic acid started to decrease after 3 days. The D (−) lactic acid reached its maximum value after 35 days before it started to decrease *(407)*. This seems to indicate that L (+) lactic acid is used before D (−) lactic acid, and that small amounts of copper may inhibit use of L (+) lactic acid more than use of the D (−) isomer. *In vitro* experiments show that this was a result of the differing influence of copper on the activities of the D (−) and L (+) forms of the lactic dehydrogenase. L (+) lactic dehydrogenase was more inhibited than the D(−) form. Lactate, especially in the D (−) form, then tended to increase in cheese paralleling the rise in copper content *(197, 198)*.

Lipolysis. Most volatile acids produced in Swiss cheese appear to be formed by the metabolism of bacteria. Schormüller *(376)* mentions that the small amounts of butyric acid found seem to come from the metabolism of clostridia. There are other sources that suggest that butyric acid and, especially, the higher fatty acids come from lipolytic breakdown of milk fat, because the proportions of free fatty acids other than propionic and acetic acid found in Swiss cheese closely resemble the proportions of fatty acids in milk fat *(36, 86, 241)*.

Milk contains a lipase that will usually be inhibited by pasteurization *(376)*. Consequently, the original lipase in milk seems of little importance in the ripening of cheese made from pasteurized milk. In Swiss cheese from raw milk and LTST heat-treated milk, however, the effect of the lipase is important *(304, 376)*. If lipase is added to the pasteurized cheese milk, lipolysis will liberate higher amounts of fatty acids than in the control vat *(304)*, so the milk lipase is not inactivated during cheesemaking as was assumed by Bachmann *(19)*.

Microbial lipases may have an important effect on cheese flavor. Generally, active lipase production is associated with psychrotrophic bacteria. Representatives of these organisms belong to the genera *Pseudomonas, Alcaligenes, Achromobacter,* and *Serratia.* Microorganisms growing on the cheese surfaces are especially active because these genera are usually strictly aerobic, but their lipase may still be effective in the center of the cheese *(376)*. Pasteurization destroys the lipase-carriers of raw milk, but the enzymes are not usually inactivated *(405)*. In a study of Swiss cheese produced from milk inoculated with lipase-producing pseudomonads and thereafter pasteurized, Pinheiro et al. *(333)* found a significant increase in

TABLE 3. ORGANIC ACIDS IN SWISS CHEESE COMPARED WITH CHEDDAR, TILSIT, AND EDAM CHEESE

Acids	Domestic Swiss[a]	Emmental[b]	Emmental[c]	Emmental[d] tear fluid	Cheddar[a]	Tilsit[c]	Edam[c]
			(mg/100 g)	(mg/10 g fluid)		(mg/100 g)	(mg/100 g)
Lactic acid	–	–	560.1	130.68	–	649.8	900
Succinic acid	–	–	123.9	95.46	–	59.3	225.7
Fumaric acid	–	–	0.58	0	–	0.29	1.45
Malic acid	–	–	25.52	13.47	–	17.1	55.2
Oxaloacetic acid	Trace	+	+	0.62	Trace	+	+
Glyoxylic acid	–	+	+	++	–	+	+
Pyruvic acid	++++	+	9.68	4.49	++++	10.74	11.76
α-Ketoglutaric acid	+++	+	1.46	1.32	++	7.67	8.40
p-Hydroxyphenyl-pyruvic acid	–	–	+	+	–	–	+
α-Ketobutyric acid	–	–	–	+	–	–	–
α-Acetolactic acid	+++	–	–	–	++	–	–

[a]Bassett and Harper *(23)*.
[b]Kreula and Virtanen *(219)*.
[c]Schormüller and Langner *(378)*.
[d]Schormüller et al. *(379)*.

free fatty acids in the cheeses that was caused by residual lipase.

No extracellular lipase has been found in lactic-acid bacteria. Nakanishi et al. *(285)* detected no appreciable lipase activity in six strains of starter bacteria, and the formation of free fatty acids in cheese was not attributed to lipase activity from starter strains. Later, however, it was shown that lactic streptococci contain intracellular lipases that hydrolyse tributyrin *(125)*. *Lactobacillus casei*, which often is present in high numbers in Swiss cheese during ripening, was shown by Peterson and Johnson *(330)* to contain intracellular lipases that are active at pH 5 to 6, covering the correct pH range for Swiss cheese during ripening. The lipases of eight strains liberated n-butyric, caproic, caprylic, and capric acids; two strains produced n-butyric, caproic, and caprylic acids *(330)*. *Lactobacillus casei* in cheese was shown to increase the relative amounts of volatile fatty acids.

The lipase activity of propionibacteria has not been studied in detail. Werner *(459)* found no extracellular lipase activity in eight strains. Cantoni et al. *(53)* demonstrated, however, that six strains could produce butyric acid from tributyrin and free fatty acids from butterfat. Oterholm et al. *(313)* established that the lipase of *P. shermanii* was an intracellular enzyme, and may have some influence on the ripening of Swiss cheese. If this surmise is correct, the effect must be minimal in view of the well-established relationship between proportionality of volatile fatty acids and desirable Swiss cheese flavor.

Production of acids other than fatty acids. Table 3 shows some of the other acids observed in Swiss cheese. The first to study the keto acids of Swiss cheese were Bassett and Harper *(22, 23)*. The content of keto acids was not essentially affected by the age of the Swiss cheese. All cheeses contained pyruvic, α-ketoglutaric, α-acetolactic, oxalosuccinic, and oxaloacetic acids with pyruvic and α-ketoglutaric as the main compounds present. In cultures of *L. bulgaricus* and *S. thermophilus*, similar patterns of keto acids were obtained but with disproportionately larger amounts of α-ketoglutaric acid. If *P. shermanii* was included in the mixed culture, the pattern corresponded to that of the cheese *(22)*. Keto acids of Swiss cheese also have been studied by Kreula and Virtanen *(219)* who detected α-ketoisovaleric acid and α-ketocaproic acid in addition to those found by Bassett and Harper *(23)*. In tear fluid, only pyruvic, α-ketoisovaleric, and α-ketoglutaric acid were found *(219)*. Schormüller and Langner *(378)* found p-hydroxyphenyl pyruvic acid in addition to the other keto acids in Swiss cheese. Lactic, succinic, fumaric, and malic acid also have been detected *(378)*. The same acids have been found in the tear fluid of Swiss cheese *(379)*.

As mentioned earlier, propionic acid usually arises from lactate, but some propionic acid may be produced from alanine, serine, or aspartic acid *(376)*. A portion of the acetic acid also could be a metabolic product of citrate breakdown by propionibacteria *(8)*. Succinic and pyruvic acid are intermediates in the breakdown of carbohydrates and lactate *(376)*. Swiss cheese older than 7 months shows no interrelations between ripening time and pyruvic acid content *(191)*. Cheeses with low copper content, however, contain less pyruvic acid than cheeses with a higher copper content. This may indicate that copper inhibits emptying of the pyruvate pool. This effect of copper will be discussed in more detail in the section dealing with diacetyl in Swiss cheese.

Amino acids are of some importance in the formation of different organic acids. Transamination and deamination of amino acids are specific enzymatic reactions of great importance. According to Virtanen and Tarnanen *(451)*, propionibacteria form aspartase, which decomposes aspartic acid into fumaric acid and ammonia. Ellfolk *(103, 104)* has isolated this enzyme and examined its mechanism. Other acids, such as α-ketoglutaric acid, α-ketobutyric acid, n-hydroxyphenyl pyruvic acid, and pyruvic acid, usually are formed by transamination of amino acids, while malic acid and oxaloacetic acid may be formed by deamination *(376)*. On the other hand, amino acids may be formed by the same reactions because these enzymatic reactions are reversible, and it is the need of the microorganism that decides which way the reaction will go. Harper *(148)* showed that amino acids were produced from keto acids by *L. bulgaricus* and *S. thermophilus*.

Protein hydrolysate in a milk medium stimulated the production of volatile fatty acids by *S. lactis (284)*. As stated in another paper, Nakae and Elliott *(282)* found that both lactic streptococci and lactobacilli produce fatty acids from casein hydrolysate. *Streptococcus diacetilactis* produces acetate from alanine, glycine, and serine, propionate from threonine; isobutyric acid from valine, and isovaleric acid from isoleucine and leucine. The reactions were ascribed to oxidative deamination *(283)*. A strain of *L. casei* produces the same volatile acids from amino acids as usually does *Streptococcus diacetilactis (283)*. *Lactobacillus casei* is found in Swiss cheese, and these reactions may be the source of some of the volatile fatty acids of Swiss cheese. Nothing is known about the production of volatile fatty acids from amino acids by propionibacteria, although Ritter and Hänni *(358)* found that isobutyric acid and isovaleric acid in Swiss cheese could be produced by micrococci from valine and leucine.

Other volatile carbon compounds

In this section, the significance of carbonyl compounds, alcohols, esters, lactones, and hydrocarbons in Swiss cheese will be discussed in detail. Little work in this field has been done with Swiss cheese, and the only detailed study was done at Oregon State University by Langler *(241)* and Langler et al. *(244)*; consequently, many of the references in this section will necessarily refer to work done with other cheeses.

Analytical methods. Few studies were attempted involving volatile carbon compounds and their importance in cheese flavors before the development of chromatographic methods, and, especially, gas chromatography. Only in the last 10 years has understanding of the importance of these compounds developed. Isolation of these compounds from cheese and other food products without loss of volatiles was so difficult that Weurman *(460)* suggested that working at subzero temperatures under N_2 atmosphere might be important to prevent chemical and enzymatic side reactions.

Volatile compounds are notable for their elusive character, their presence in minute amounts, and their unstable nature. They, therefore, present the analyst with a considerable problem in isolation and identification as unaltered flavor components. Usually, techniques such as solvent extraction and atmospheric or vacuum distillation are used before gas chromatography, but, recently, direct analysis of cheese or foods by "head-space vapor analysis" or "on-column trapping" has been used *(250, 275, 460)*.

Steam distillation of cheese slurry was one of the first methods tried and was used by Walker and Harvey *(454)*, who estimated amounts of n-saturated aldehydes and ketones up to C_{11} in Cheddar cheese by reaction with 2, 4-dinitrophenylhydrazine in an acid solution under anaerobic conditions *(246)*.

The usual method is to isolate cheese fat, containing the major portion of the volatiles, by centrifuging a mixture of cheese and water at about 40 C (104 F) *(75, 252)*. The isolated fat is then either extracted or distilled. A recycling gas-liquid extraction apparatus in which a sweeping gas is recycled and pushes volatiles into a refrigerated trap has been developed *(288)*. Another method using countercurrent contact of the milk fat with steam in a column, followed by ether extraction of the condensed steam also has been used *(54)*. The usual methods, however, use either a simple vacuum distillation method, which is especially effective for aldehydes *(247)*, or a high-vacuum apparatus for isolation of all volatiles *(50)*. A low-temperature low-pressure distillation for volatiles in Cheddar cheese has been used *(250)*, and Langler et al. *(244)* used the same method for Swiss cheese. In this method, flavor volatiles are distilled into liquid nitrogen traps of glass, which can be fitted with a hypodermic needle that inserts the sample into the gas chromatograph. For identification, the different chromatographic peaks may be isolated by a trap and then used for mass spectral analysis *(250, 271)* or admitted directly to the rapid-scan mass spectrophotometer *(75)*. Direct injection of the cheese oil into the gas chromatograph also has been used for analysis of cheese volatiles *(252)*.

Extraction also may be used for isolation of volatile compounds before gas chromatography. For example, finely grated Cheddar cheese has been mixed with Celite 545 and packed into a column that was eluted with redistilled acrylonitrile *(466)*. The first few drops were sufficiently concentrated to permit gas-chromatographic analysis without solvent evapora-

tion. Recovery of added benzoic acid was 103%. Liebich et al. *(253)* determined the volatile compounds in Cheddar cheese by using different isolation methods: *(a)* centrifuging to separate the oil from the cheese, *(b)* low-temperature vacuum distillation of the cheese followed by extraction of relevant fractions with diethyl ether, *(c)* treatment of whole cheese as in *(b)*, and *(d)* extraction of cheese oil with methanol. Oil from *(a)* and extracts from *(b)*, *(c)*, and *(d)* were analyzed by gas-liquid chromatography, and extracts were further analyzed for identification of compounds by combined gas-chromatography-mass spectrometry. More than 150 components were found by gas chromatography of Cheddar cheese, of which 120 were identified.

Large amounts of cheese are needed for distillation and extraction methods. The analysis of head-space gas samples, on the contrary, uses rather small samples. A method consisting of drawing gas from a column into the body of a hypodermic syringe containing cheese between Lintine filter discs, sealing the needle, heating to 90 C (194 F) for 8 min, and injecting the mixture of gases, including low boiling components into the column has been described *(227)*. The method was empirical but fairly reproducible, and was used to compare the peak profiles of 15 different cheeses each of which showed a different profile. Injection of volatiles in the form of head space vapor into the column facilitated analysis and rendered less liable decomposition of the volatile components *(317)*. Morgan and Day *(275)* felt that the headspace vapor method lacked precision and used the technique of on-column trapping of entrained volatiles, as described by Hornstein and Crowe *(168)*. Samples were treated with sodium sulfate to saturate the aqueous portion, and the volatile matter was entrained with 50 to 100 ml nitrogen at 60 to 90 C (140 to 194 F), which gave sufficient material to detect minor amounts. This method was used by Langler for analysis of volatiles in Swiss cheese *(241)*.

Besides the gas chromatographic method, colorimetric methods for specific compounds have been developed including a method to determine carbonyl compounds by use of dinitrophenyl hydrazines *(279)*. Different colorimetric methods have been developed for diacetyl *(56, 172, 249, 316, 396)*. Methods for formaldehyde and acetaldehyde also have been worked out *(258, 273, 428)*.

Volatiles and production of volatiles in Swiss cheese. Before Langler's work in 1966 *(241, 243, 244)* very little research had been done in regard to volatiles in Swiss cheese. Acetoin and diacetyl were found in Swiss cheese in 1941 by Csiszar et al. as reported by Tomka *(435)*. In 1958, Bassett and Harper *(23)* isolated these compounds from Swiss cheese: acetaldehyde, acetone, 2-pentanone, diacetyl, acetyl-methyl carbinol, and a five-carbon and a seven-carbon aldehyde or ketone. As shown in Table 4, Langler *(241)* identified 66 compounds in Swiss cheese. The amounts of some selected compounds are shown in Table 5 *(244)*. These compounds will be discussed by the groups to which they belong:

TABLE 4. VOLATILES FOUND IN SWISS CHEESE *(241)*

Alcohols
1. Ethanol
2. 1-Propanol
3. 1-Butanol
4. 2-Pentanol
5. *trans*-2-Hexene-1-ol
6. 2-Phenylethanol
7. 2-Butanol

Aldehydes
1. Acetaldehyde
2. Propanal
3. Butanal
4. 2-Methylpropanal
5. 2-Methylpropenal
6. 2-Methylbutyraldehyde
7. Benzaldehyde
8. Phenylacetaldehyde

Esters
1. Ethyl formate
2. Methyl acetate
3. Ethyl acetate
4. Ethyl propionate
5. Ethyl butanoate
6. Butyl acetate
7. 3-Methylbutyl acetate
8. Methyl hexanoate
9. Ethyl hexanoate
10. Methyl benzoate
11. Methyl octanoate
12. Ethyl octanoate
13. Methyl decanoate
14. Ethyl decanoate
15. 3-Methylbutyl octanoate
16. Ethyl dodecanoate

Hydrocarbons
1. Toluene
2. 1-Octene
3. Nonane
4. Ethyl benzene
5. 1,2-; 1,3-; or 1.4-Dimethylbenzene
6. Nonene
7. Dodecane
8. 2-Phenyl-2-methylbutane
9. 5-Methyl-5-ethyldecane
10. 2,5-Dimethyltetradecane
11. Pentadecane

Lactones
1. γ-Valerolactone
2. δ-Octalactone
3. δ-Decalactone
4. γ-Dodecalactone
5. δ-Dodecalactone

Methyl ketones
1. Acetone
2. Butanone
3. 2-Pentanone
4. 2-Hexanone
5. 2-Heptanone
6. 2-Nonanone
7. 2-Undecanone
8. 2-Tridecanone
9. 2-Pentadecanone

Miscellaneous compounds
1. Diacetyl
2. Dimethyl sulfide
3. Methyl vinyl ether
4. Chloroform
5. α-Pinene
6. α-Fenchene
7. o-Dichlorobenzene
8. 1,2,4-Trichlorobenzene
9. Benzothiazole
10. Diisobutyl adipate

I. *Alcohols.* Usually the amounts of alcohols found in Swiss cheese are too low to be believed to have any direct influence on flavor. Indirectly, however, they may contribute to flavor because of their ability to form esters with fatty acids. It should be mentioned that propyl esters have not been found *(241)*. Major alcohols present are ethanol and 1-propanol. Ethanol is a common terminal endproduct

TABLE 5. SELECTED COMPOUNDS IN SWISS CHEESE VOLATILES IN PPM *(244)*

Compound	Selected volatiles in Swiss cheese					
	Domestic Swiss				Emmental	
	A	B	C	D	E	F
Dimethyl sulfide	0.106	0.056	0.123	0.183	0.079	0.094
Diacetyl	0.8	0.2	0.6	0.4	1.4	1.4
Acetaldehyde	2.0	0.6	1.8	1.8	1.1	1.0
Acetone	0.6	0.6	2.1	0.7	1.6	3.9
Butanone	0.7	0.1	0.1	0.1	0.2	0.6
2-Methylbutyraldehyde	0.06	0.06	0.09	0.1	0.3	0.2
2-Pentanone	0.06	0.09	0.06	0.09	1.36	4.24
2-Heptanone	0.06	0.15	0.12	0.12	0.73	1.49
Ethanol	9.6	3.5	35.3	24.9	16.7	8.0
2-Butanol	0.5	0.1	0.1	0.1	0.7	1.0
1-Propanol	2.1	0.8	4.1	3.5	2.7	1.1
1-Butanol	0.8	1.1	1.1	0.6	0.4	0.2
Methyl hexanoate	4.1	0.3	1.8	0.8	1.7	0.1
Ethyl butanoate	0.1	0.1	1.0	1.0	0.2	0.2

in the breakdown of glucose *(72)*, but the mode of formation of the methanol that has been found is hard to explain. Keenan et al. *(187)* demonstrated that *S. lactis*, *S. cremoris*, and *S. diacetilactis* produce ethanol, although *S. diacetilactis* produces it only in small amounts. These results were confirmed by Bassette et al. *(25)* for *S. lactis*, but not for *S. diacetilactis*. The same workers *(25)* also found that *L. acidophilus* produces ethanol. 1-Propanol and 1-butanol might be expected to result from reduction of their corresponding aldehydes. *Lactobacillus brevis*, *L. casei*, *Lactobacillus plantarum*, and *L. lactis* have been observed to produce ethanol and 1-propanol from acetaldehyde and propionaldehyde *(185)*. *Lactobacillus brevis* also produces 2-butanol from butanone. A strain of *L. brevis* produced 1-propanol as a normal metabolite in cheese. Three strains of *P. shermanii* were shown to produce ethanol and 1-propanol *(183)*. The reduction of 2-pentanone to 2-pentanol by Swiss-cheese starter organisms has never been observed, but mold and yeasts may reduce 2-pentanone to 2-pentanol *(6)*. The film yeasts formerly used with Swiss-cheese starters may have been a source of 2-pentanol *(116)*. Trans-2-hexene-1-ol was found in Swiss cheese. The source for this may be trans-2-hexenal, which has been found in the grassy aroma constituents of green forage *(277)*. This compound may be reduced to the alcohol during ruminant metabolism and, thereafter, transported to the mammary gland. Another possibility for production would be by autoxidation of linolenate *(277)*. 2-Phenylethanol also has been found in Blue cheese *(73)*, and this compound imparts desirable character to a synthetic blue cheese flavor mixture *(241)*. This compound may possibly be formed via degradation of phenylalanine and may be of some importance in Swiss cheese flavor.

II. *Aldehydes.* Acetaldehyde is the most common aldehyde found in fermented dairy products and cheeses *(38, 74, 257, 286, 454)*. Acetaldehyde as a common carbonyl compound in Swiss cheese was first established by Bassett and Harper *(23)*. The average concentration in Swiss cheese is close to 1.4 mg/kg *(241)*. The flavor threshold of acetaldehyde is 0.4 mg/kg *(151)*. Amounts of acetaldehyde in Swiss cheese *(241)* have been compared with amounts found in good-flavored butter cultures *(257)* and, on this basis, it has been assumed that acetaldehyde is an important factor in Swiss-cheese flavor.

Acetaldehyde is produced chiefly by bacterial metabolism. Production of acetaldehyde by 11 strains of *S. lactis* and *S. cremoris* was established by Harvey *(151)*. No other aldehydes were formed. *Streptococcus lactis*, *S. lactis* var. *maltigenes*, *S. cremoris*, and *S. diacetilactis* produce amounts of acetaldehyde well over the flavor threshold in whole-milk media, and excessive amounts of acetaldehyde result in "green" flavor *(260)*. Some of the acetaldehyde produced, however, would be removed by prolonged incubation *(187)*. It is claimed that *Streptococcus thermophilus* produces more acetaldehyde than other homofermentative streptococci *(44)*. *Streptococcus thermophilus* also produces acetaldehyde under anaerobic conditions *(417)*.

Lactobacillus casei grown in milk produces acetaldehyde, but after 4 days the acetaldehyde disappears *(25)*. *Lactobacillus casei*, *L. brevis*, and *L. plantarum* produce acetaldehyde slowly at temperatures close to cheese ripening temperatures *(185)*. Three strains of *P. shermanii* have been shown to produce acetaldehyde and propionaldehyde *(169)*. Different strains of starter organisms may produce different amounts of acetaldehyde, which may be the cause of off-flavors produced by some starters *(409)*.

The other aldehydes found in Swiss cheese seem of less importance as flavor compounds. The branched chain aldehydes may possibly be intermediates in the production of fatty acids from amino acids *(260)*. Phenylacetaldehyde may be formed by deamination and decarboxylation of phenylalanine. Benzaldehyde and phenylacetaldehyde exist in the volatiles of grass and corn silage, which may be another explanation of their occurrence in cheese *(257)*.

III. *Esters.* Ethyl butanoate and methyl hexanoate are the primary esters found in Swiss cheese. Esters may influence the flavor of cheese, and if they are found in amounts higher than their threshold value, a fruity flavor may be recognized *(37)*. The presence of esters in Swiss cheese is assumed to be due to the existence of free fatty acids and alcohols *(241)*. Whether they are formed by simple mass action or enzymatic reactions is unknown.

IV. *Hydrocarbons.* Aromatic hydrocarbons have been found in Cheddar and Blue cheese, but not alkyl hydrocarbons, which in Swiss cheese possibly arise from packing material *(241)*. Toluene also has been found in Swiss cheese, and did not seem to be an artifact introduced by distillation. The terpene, α-pinene, although present, was probably introduced into the milk from the forage eaten by the cow. None of the hydrocarbons seem important for flavor.

V. *Lactones.* δ-Lactones and γ-lactones were found in both Swiss and Cheddar cheese *(241, 305)*. The concentration of lactones in Swiss cheese is very low and probably of no importance in flavor. Lactones are normal constituents of milk fat *(40)*. δ-Lactones also may be produced by heating of milk *(375)*. γ-Valerolactone probably is not a normal constituent in milk. It may be produced by microorganisms, and Boldingh and Taylor *(40)* mention various microorganisms that may convert keto-acids to corresponding lactones.

VI. *Methyl ketones.* A homologous series of methyl ketones was found in Swiss cheese *(241)*. Their concentrations varied considerably between different cheeses. The presence of methyl ketones in heat-treated milk fat was determined by Langler and Day *(242)*. The same compounds have been found in heat-treated milk, dry whole milk, and evaporated milk *(320, 375, 467)*. These compounds could, therefore, be expected in Swiss cheese that is cooked at a high temperature *(72)*. For example, methyl ketone concentrations have been reported to be highest in imported Swiss cheese, which might imply a higher cooking temperature or a longer cooking period *(241, 244)*. Milk fat contains sufficient precursor to explain the level in Swiss cheese, and water is necessary for methyl ketone formation. The 2-heptanone level seems quite close to the threshold value. Methyl ketone mixtures have been observed to have a synergistic effect *(242)*, so it is probable that methyl ketones affect the flavor of Swiss cheese *(241)*.

Other sources of methyl ketones in microbial metabolism may not be ruled out. Methyl ketones are produced by molds used for Blue cheese production *(72)*, but the starter organisms used for Swiss cheese have never been observed to produce methyl ketones. Development of methyl ketones in Cheddar cheese has been followed by Harvey and Walker *(152)*. In 1-day-old cheeses, they found acetone, 2-butanone, and 2-pentanone; after 2 to 4 weeks, 2-heptanone was found, and after 20 weeks, 2-nonanone. The flavor of mature cheese was apparent after 8 to 12 weeks with an increase in 2-pentanone and 2-heptanone. This may indicate microbiological production of methyl ketones. Seven strains of S. *lactis* and S. *cremoris* have been shown to produce acetone *(151)*. Production of acetone in milk cultures by S. *lactis*, S. *cremoris*, and S. *diacetilactis* has also been reported *(184)*. Reports of the production of methyl ketones by high-temperature lactic-acid bacteria or propionic-acid bacteria could not be found.

VII. *Miscellaneous compounds.* The major portion of this discussion will be concerned with diacetyl. Some compounds found in minor amounts in Swiss cheese by Langler *(241)*, however, deserve prior mention. Benzothiazole was recovered from Swiss cheese. Chlorinated aromatic compounds also were isolated but they might have been solvent contaminants. They also may originate from herbicides. Chloroform and di-isobutyl adipate also were isolated from Swiss cheese.

Csiszar et al. *(64)* found an average of 2.6 mg diacetyl/kg Swiss cheese, but they did not observe any relationship between this compound and the flavor of Swiss cheese. An average of only 0.8 mg/kg in Swiss cheese, however, was found by Langler *(241)*. The threshold value of diacetyl is dependent on the medium in which it exists *(27, 155)* and the level of diacetyl in Swiss cheese is well over the threshold value and is considered an important part of the flavor *(241)*.

Diacetyl is usually regarded as a by-product of citrate fermentation or it also can be obtained from carbohydrate metabolism. A scheme for the synthesis of diacetyl from citric acid has been prepared by Lindsay *(257)*. Alpha-acetolactic acid was shown to be an essential intermediate in the synthesis of diacetyl *(22, 181, 457)*, and pyruvate is an intermediate.

In cheese containing copper, the amount of pyruvate increased with the amount of copper in the cheese *(191)*. The amount of diacetyl and acetoin also decreased with increasing amounts of copper

(192). The enzyme pyruvate decarboxylase is inhibited *(193)*, and it seems that copper inactivates the participation of thiamine in the decarboxylation of pyruvate *(194)*. Acetoin and diacetyl are formed by two different enzymes, and Collins *(62)* showed that acetoin could be formed from diacetyl but the opposite reaction does not occur. The earlier belief that this reaction was reversible *(240)* was shown to be incorrect *(62)*.

The microbial flora of Swiss cheese is complex, and diacetyl could be produced by the heterofermentative species in mixed-strain lactic cultures *(141)*. *Streptococcus thermophilus* was found to produce more diacetyl than other homofermentative lactic streptococci *(44)*. The enzyme diacetyl reductase, which produces acetoin is found in S. *lactis* and S. *cremoris (388)*. Keenan and Lindsay *(186)* found that *L. casei* and *L. plantarum* accumulated detectable amounts of diacetyl at 8 C (46.4 F). Branen and Keenan *(45)* observed that *L. casei* contained the enzyme diacetyl reductase. *Lactobacillus casei* showed the greatest ability to convert pyruvate to diacetyl and acetoin during late logarithmic growth; the optimum pH for diacetyl and acetoin production was in the range 4.5 to 5.5, which indicates that this conversion may take place in cheese *(47)*.

Two strains of rod-shaped propionibacteria that produced acetoin and diacetyl in sterile milk and sterile whey were found by Tomka *(435)*. The maximum level of acetoin was noted during the first few days of growth and remained constant for 15 days. A comprehensive study of acetoin and diacetyl production by propionibacteria was made by Antila *(10)* who found that these compounds were produced from pyruvic acid with α-acetolactic acid as an intermediate, as for other diacetyl-producing bacteria. In a detailed study by Lee et al. *(248)*, a strain of *P. shermanii* was found that produced relatively large amounts of diacetyl. The diacetyl accumulation was greater at 21 C (69.8 F) than at 32 C (89.6 F) or 37 C (98.6 F), although the production was faster at the higher temperatures. Rapid cooling after incubation stabilized the level of diacetyl. From pH 4.0 to 4.5 was the most favorable range for diacetyl biosynthesis. Little or no diacetyl reductase was found in *P. shermanii (248)*.

According to Emilsson and Sjöström *(106)*, propionibacteria cannot ferment citric acid in cheese, however, these observations may have been due to the slow growth of the specific strain used in the study *(8)*. Hietaranta and Antila *(164)* observed that high-grade Swiss cheese contained only traces of citric acid and that citric acid disappeared during maximum growth of propionibacteria. In another report, Hietaranta and Antila *(163)* reported that *P. peterssonii* fermented citric acid more rapidly than *P. pentosaceum* and that the utilization of citric acid was repressed by lactic acid.

Nitrogen compounds

Considerable attention has been devoted to the importance of amino acids in Swiss cheese. Some workers attribute major significance in flavor production in Swiss cheese to amino acids. Others regard the influence of amino acids on Swiss cheese flavor with greater reservation. Amino acids, doubtless, have an influence on cheese flavor, and a high glutamic-acid content has been shown to have a direct influence in this regard *(147)*. Even peptides seem to have taste-forming influences *(414)*. Proteins, on the contrary, induce no flavor but are important for the body and texture of cheese. In this section, amino-acid content and its influence on flavor and the biochemical production and degradation of amino acids in Swiss cheese will be discussed.

Amino acids in Swiss cheese. Virtanen and Kreula *(449)* studied the relation of Swiss cheese flavor to the amino acids present in cheese and found that proline and hydroxyproline had a sweet taste and constituted a high proportion of the amino acids in the cheese serum. They, therefore, concluded that proline might be the cause of the sweet flavor. The high arginine content of casein also was thought to be another factor influencing flavor, but during ripening, 66% of the arginine was lost, so the disagreeable flavor of arginine had no effect *(449)*. Two-thirds of the nitrogen compounds of tear fluid of Swiss cheese consisted of free amino acids and one-third of peptides and proteins. Proline was abundant in the tear fluid *(450)*. Storgårds and Hietaranta *(413)* considered that a portion of the flavor was due to the higher amounts of glutamic acid and aspartic acid in Swiss cheese than in other hard, rennet cheeses. The amount of acidic amino acids increased and the amount of basic amino acids decreased during storage of Swiss cheese.

The first qualitative study of amino acids in Swiss cheese was done by Block *(39)* in 1951. All the amino acids present in casein, as well as different degradation products of amino acids, were found using paper chromatography. Storgårds and Lindqvist *(414, 416)* also studied the amino acids of cheese quantitatively. They determined that the amount of proline in Swiss cheese was higher than in any other cheese. The quantitative amount of amino acids in Swiss cheese was determined by paper chromatography by Kosikowski and Dahlberg *(214)* and Hintz et al. *(165)*. Antila and Antila *(15)*, and Ritter et al. *(361)* measured the amino acid content using more

TABLE 6. FREE AMINO ACIDS IN SWISS CHEESE COMPARED WITH THOSE IN GRUYERE, EDAM, AND CHEDDAR CHEESE (MG/100 G CHEESE)

	Free amino acids in cheese								
	Swiss cheese, domestic[a]				Emmental[b]				
Amino Acid	No flavor	Mild	Medium	Pronounced	3 month	6 month	Gruyère[b]	Edam[b]	Cheddar[b]
Aspartic acid	260	Trace	0	160	9.2	16.7	32.9	2.1	37.2
Threonine					42.0	68.9	78.1	14.5	90.0
Serine					32.2	54.9	44.6	7.1	67.9
Glutamic acid	87	210	330	180	134.6	268.1	296.4	35.2	273.4
Proline	30	350	300	530	134.4	253.5	281.7	15.4	54.4
Glycine	0	90	120	190	16.5	43.1	49.3	3.5	32.6
Alanine					36.4	56.8	59.8	6.9	53.8
Valine					86.9	156.2	181.8	16.7	146.9
Methionine					28.1	50.3	53.2	6.0	67.6
Isoleucine					52.2	105.1	118.9	4.8	101.4
Leucine					117.0	179.5	177.9	42.6	190.1
Tyrosine					4.3	28.6	32.1	8.9	29.7
Phenylalanine					73.0	127.9	136.7	29.1	132.7
γ-Amino butyric acid					10.0	26.1	30.8	1.2	37.0
Ornithine					74.5	113.3	99.3	22.4	140.0
Lysine	230	150	100	950	146.5	222.0	222.8	24.6	216.2
Tryptophan	72	Trace	54	64	4.5	2.2	12.0	—	6.6
Histidine	0	350	110	0	51.2	86.8	68.1	5.0	58.2
Arginine					3.5	1.9	2.1	13.1	7.8
Tyrosine + phenylalanine	190	390	540	290					
Threonine + serine	190	170	130	450					

[a]Hintz et al. *(165)*.
[b]Antila and Antila *(15)*.

exact ion-exchange chromatographic systems. The amino acid content of Swiss cheese tear fluid was measured by Schormüller et al. *(379)*. These results are found in Table 6 and are compared with the amino-acid content of Edam and Cheddar cheese.

Not all amino acids are present in various Swiss cheeses and amounts vary widely among cheeses *(165)*. Proline showed the widest quantitative variation, from 0 to 5.8 mg/g cheese. Amounts of cysteic acid, taurine, proline, lysine, and histidine tended to increase during storage, but the amount of glycine tended to be constant. Amounts of aspartic acid, threonine, serine, glutamic acid, tyrosine, phenylalanine, and tryptophan present could not be related to the age of the cheese. A relationship between proline and propionic-acid content and flavor was believed to exist although Jäger *(175)* found no correlation between amino acids and flavor. The work of Antila and Antila *(15)* and Ritter et al. *(361)* showed that there were distinct differences between Finnish and Swiss Emmentaler. The proportion of glutamic acid to proline in Swiss Emmentaler was 2:1; in the Finnish cheese, it was 2:3. The amount of arginine was 40 times higher in Swiss Emmentaler. These differences may be due to different manufacturing processes and different strains of starters used.

The sweet taste attributed to proline in Swiss cheese may also be due to the group of sweet amino acids: glycine, alanine, proline, serine, and threonine, but the proline content is much higher than any of the other sweet amino acids. The ratio of bitter amino acids to sweet amino acids was 2.33 in poor-quality Swiss cheese, but in high-grade Swiss cheese, it was 1.93 to 2.05 in studies by Dylanyan et al. *(99)*. Schormüller et al. *(379)* found that the amino-acid composition of the tear fluid of Swiss cheese was qualitatively, but not quantitatively, equal to that of casein. Proline content was about twice as great in the tear fluid.

Production of peptides and amino acids. Rennet coagulation of Swiss cheese consists of two reactions: (a) A primary reaction with liberation of nonprotein nitrogen from the casein for curd production, which is a very fast reaction. *(b)* A secondary reaction that starts the proteolytic degradation of the caseins *(376)*. This proteolysis of casein has been followed by electrophoretic studies. Degradation of casein proceeds in different ways according to cheese types as shown by Lindqvist and Storgårds *(254)*. Hard cheeses as Svecia and Swiss cheese had an "alpha-ripening," which means that the greatest change took place around the α-casein peak, but cheeses such as Port Salut and Camembert, showed a "beta-ripening." Instances of nonspecific casein degradation,

as in Tilsit and Limburg, also were found. During ripening of Svecia cheese, Lindqvist and Storgårds *(255)* found that the first stage was decomposition of a component of the α-casein peak, probably k-casein. Approximately at the same time, degradation of the β-casein takes place. α-casein is not attacked until after a longer period of ripening. The same changes were observed with Swiss cheese *(325)* but Dylanyan et al. *(99)* do not believe that this division of the ripening process into different categories is justified.

This degradation of caseins could be caused by rennet, the natural proteinase of milk, and microbial proteinases but Stadhouders *(404)* found that the proteolytic activity of rennet and the milk enzyme was of only minor importance during cheese ripening and that the main enzymes were microbial proteinases. Poznański and Rymaszewski *(327)* studied the proteolytic activity of S. *thermophilus* and *L. bulgaricus* in Edam cheese ripening. Addition of *L. bulgaricus* gave a satisfactory flavor, better than S. *thermophilus*, although addition of *Micrococcus caseolyticus* gave a bitter cheese *(337)*. Only the endoenzymes of *L. bulgaricus* acted appreciably on whole casein, and those from S. *thermophilus* and *M. caseolyticus* had greater effects on intermediate products from casein. The activity of the bacterial enzymes was found to be low compared with the activity of rennin, and the effect of combined enzymes was greater on α_r-casein and k-casein than on whole casein *(336)*. The enzymes produced by S. *thermophilus* and *L. helveticus* decomposed, to a varying extent, para-casein degradation products formed as a result of rennet hydrolysis *(335)*. Proteinases of lactic-acid bacteria seem to play a particular role in flavor development. Rapp *(342)* observed that *L. acidophilus* showed the highest proteolytic activity followed by *L. bulgaricus*, *L. helveticus*, and *L. casei*, in that order. Dyachenko et al. *(96)* observed that 11 thermophilic *Lactobacillus* strains showed higher activity than 3 S. *thermophilus* strains. *Lactobacillus helveticus* showed the highest activity. Since strain differences within species can sometimes be greater than between representatives of different species, ratings of this nature are risky at best. Studies by Ohmiya and Sato *(301)* indicated that the extent of casein hydrolysis by intracellular proteases of S. *cremoris* and *L. helveticus* is similar to that of rennin. Aseptic rennet curd and aseptically harvested and washed cells of S. *cremoris* and *L. helveticus* were sandwiched between aseptic rennet curd. The curd was then coated with wax and incubated. Rapid autolysis of the cells occurred *(302)*. The nonprotein nitrogen content increased, even in the aseptic curd during ripening at 10 C (50 F), but the increase was much smaller than in aseptic curd inoculated with S. *cremoris* and *L. helveticus (303)*. This indicates that rennet is more active than Stadhouders *(404)* assumed. α_s-Casein was most easily degraded by the lactic acid bacteria. On the contrary, Dyachenko and Shidlovskaya *(97)* observed that *L. bulgaricus*, *L. helveticus*, S. *cremoris*, and S. *thermophilus* decomposed β-casein more rapidly than α-casein. The methods used to measure proteolytic activity also are important. *Lactobacillus thermophilus* showed much greater activity when assayed by measurement of tryptophan and tyrosine than by formol titration, although the reverse was true for S. *thermophilus (300)*. Proteolytic enzymes have not been found in propionibacteria *(28, 207)*.

The products of the proteolysis of the caseins are peptides. Schormüller and Belitz *(377)* showed that during the first 14 days of the ripening of Harz cheese that the amino acid content increased and then decreased to the 21st day. The amounts of peptides increase progressively during this period. In all cheeses investigated a characteristic peptide typical for casein degradation was isolated. Using electrophoresis, Storgårds and Lindqvist *(415)* investigated the peptide distribution of Svecia, Herrgård, Swiss, Port Salut, Blue, and Camembert cheese and found that all had a characteristic peptide composition. By more detailed studies it may be possible to isolate the different peptides and obtain a more exact picture of the differences in proteolysis between different cheeses.

The best known flavor influence of peptides is, of course, that they may produce a bitter flavor, which is considered a defect in Swiss cheese. But usually during ripening, the peptide content decreases *(415)*. Since many of these peptides are phosphopeptides, which are not attacked by peptidases, phosphatases must remove the phosphates before the peptide can be degraded to amino acids. Phosphatases have been found in cheese, but because of their low activity in ripening cheeses, they may be the limiting factor for peptide degradation *(376)*.

Peptidases are found in many microorganisms, but they have not been studied as much as the proteinases *(376)*. Bipeptidases, amino peptidases, and carboxypeptidases were found in sour-milk cheese *(383)*, which showed the same pH-maximum as peptidases of microbial origin. The peptidases in the cheese were quite similar to the enzymes found in yeasts, bacteria, and animal tissues *(376)*. Peptidases also have been found in propionibacteria, and these enzymes have an optimum pH close to 5.5 to 6.0 *(28)*, which may be of importance in Swiss cheese. Schormüller and Müller *(380, 381)* were especially interested in the enzymes prolinase and prolidase in sour-milk cheese because free proline has always

been found in cheese. Neither prolinase nor prolidase are found in the cheese or in the fresh curd, but they develop during ripening from the outside to the inside. Their highest activity in sour-milk cheese was found close to the rind, and the activity decreased toward the core. Prolidase activity also has been found in Swiss cheese *(381)*.

The peptides formed during casein decomposition may produce a bitter taste in the cheese if certain microorganisms are deficient in the peptidases that participate in peptide degradation *(376)*. Stadhouders *(404)* found that bitter cheese most frequently developed at pH 5.2. The starters that decompose bitter polypeptides may differ entirely in characteristics from the nondecomposing starters. This capability was specifically marked for some strains, and it was not connected with the capability to liberate free amino acids.

This may be because a pyrrolidone carboxylic acid at the N-terminal end of a hydrophobic peptide derived from casein may cause the bitterness in some instances *(108, 420)*. It was suggested by Sullivan and Jago *(420)* that removal of bitterness by non-bitter starters was due to the presence of a pyrrolidone carboxyl peptidase; a view that was opposed by Exterkate and Stadhouders *(108)*, who found this enzyme in higher amounts in bitter than in non-bitter strains of *S. cremoris*.

The amino acids produced by this process will be more or less decomposed or rebuilt by microorganisms in the cheese. Changes in the amino acid spectrum of milk induced by the growth of two strains of *S. thermophilus* and four strains of *Lactobacillus* have been recorded by Grudzinskaya and Koroleva *(140)*. Amino acids present in considerable amounts in milk were glutamic acid, glycine, threonine, alanine, tyrosine, methionine, and valine. Streptococci removed almost all of these amino acids during their early stages in milk. Growth of *L. bulgaricus* and *L. acidophilus* left the glutamic content almost unchanged, reduced that of threonine, and led to accumulation of histidine, arginine, tyrosine, phenylalanine, leucine, cysteine, methionine, and valine. Combinations of streptococci and lactobacilli intensified both utilization of amino acids present and formation of others. It has been observed by Kiuru et al. *(204)* that, during eye formation in Swiss cheese, the quantity of certain amino acids either is constant or declines. After 90 days, some amino acids increase greatly, but others decrease. Free aspartic acid disappears probably because of aspartase, which has been found in propionibacteria *(451)*. After 45 days, the arginine content also is close to zero. Alanine, valine, serine, tyrosine, aspartic acid, glutamic acid, arginine, cystine, and methionine are degraded by propionibacteria *(9)*.

The most important degradation products from amino acids with reference to flavor are probably amines, which are produced by decarboxylation of amino acids. The following amines have been found in Swiss cheese: tyramine *(213)*, histamine *(440)*, γ-aminobutyric acid and cadaverine *(39)*, and putrescine *(426)*. The hard cheeses seem generally to have low amounts of these amines *(376)*. The amino-acid decarboxylases occur only sporadically in lactobacilli *(376)*, and Dacre *(68)* found only one strain of *Lactobacillus* with a high amount of tyrosine decarboxylase. Amino acid decarboxylase activity has not been reported for propionibacteria. The tyramine content found in cheese has been attributed by Kosikowski and Dahlberg *(213)* to tyrosine decarboxylase produced by streptococci, especially *S. faecalis*. Other disadvantageous bacteria, such as coliforms, also may produce these compounds *(376)*.

Serine and threonine may be decomposed to α-amino butyric acid and α-alanine in sour-milk cheese according to Schormüller *(376)*. Arginine, which has a repulsive, bitter, sweet taste, was shown by Schormüller and Tänzler *(382)*, to be decomposed via citrulline and ornithine to putrescine by arginine desimidase and is, therefore, eliminated as a flavor-diminishing amino acid. Transamination reactions also take place during the metabolism of amino acids.

Sulfur-containing compounds

These compounds must usually originate from the sulfur-containing amino acids: cysteine, cystine, and methionine. Formation of hydrogen sulfide has been investigated most intensively. Few microorganisms seem to deaminate sulfur-containing amino acids, but biological hydrogen sulfide formation from cystine and cysteine has been proven for *Escherichia coli (61)*. Similar possibilities also have been observed for methionine *(127)*. Desnuelle and Wookey *(83)* noted that propionibacteria liberated hydrogen sulfide rapidly from cysteine. Lactic acid activated this liberation. Desulfhydrase activity has been observed in Cheddar cheese *(55)*. It is understandable, therefore, that hydrogen sulfide always occurs in cheese—although in small amounts. Kristoffersen *(220)* and Kristoffersen et al. *(225)* showed that concentrations of hydrogen sulfide and fatty acids were related to the flavor of Cheddar cheese although Kristoffersen *(221)* also observed sulfhydryl groups in Swiss cheese and found that they were not related to the cheese quality. The occurrence of active sulfhydryl groups, however, seems to coincide with the onset of eye formation *(222)*. Redox-potential and copper content seemed to have no effect on formation of active sulfhydryl groups, but the rate of acid

development affected the concentration of sulfhydryl groups.

Besides hydrogen sulfide, mercaptans, thioethers, and dialkyl-disulfide have been found in cheese *(376)*. Studies by Purvis et al. *(341)* with ^{35}S-labelled milk showed that radioactive sulfur compounds were formed in Cheddar cheese. Methyl mercaptan was isolated from Cheddar cheese by Libbey and Day *(251)*. The amounts of mercaptans, thioethers, and dialkyl-disulfides were found in larger amounts in Cheddar cheese made from raw milk than from pasteurized milk *(224)*. Methyl mercaptans were found to be important flavor compounds in soft cheeses, as Limburger and Romadur *(180)*, and also in semisoft cheeses, as Port Salut and Trappist cheese *(138, 139)*, but they seem of little importance in Swiss cheese. But, sometimes, an undesirable influence of sulfur compounds on the taste and odor of Swiss cheese may occur. A German patent by Karrer *(182)* attempts to eliminate this off-flavor (which may be due to formation of sulfur compounds during the in vat treatment in steel vats) by suspending a copper plate in the vat. An electrolytic effect is produced, and the sulfur compounds are deposited on the plate.

The most important sulfur-containing compound in Swiss cheese is dimethyl sulfide *(241)*, which may be an important contributor to Swiss cheese flavor. Dimethyl sulfide was found in amounts from 0.056 to 0.183 mg/kg in Swiss cheese *(241, 244)*. Dimethyl sulfide was found to be a normal constituent of milk and was found to contribute to the milk flavor *(324, 343)*. The amount of dimethyl sulfide found in Cheddar cheese was much lower than in Swiss cheese and was expected to have no influence on flavor *(241)*. The flavor threshold in bland milk fat was 0.024 mg/kg, and dimethyl sulfide constitutes the fresh bouquet of butter *(76)*. The concentration of dimethyl sulfide was about four times this value in Swiss cheese *(241)*. Part of the dimethyl sulfide content in Swiss cheese may be produced by chemical degradation of methional *(72)*, but it is generally believed that this compound is produced by microbial metabolism. A strain of *Enterobacter aerogenes*, which caused a cowy flavor in milk due to dimethyl sulfide, was found to produce dimethyl sulfide in amounts that caused this flavor *(434)*. In a study of the production of volatile compounds by *P. shermanii*, Keenan and Bills *(183)* found a strain that produced dimethyl sulfide in quantities significant from the standpont of flavor. This strain was found by Dykstra et al. *(98)* to produce dimethyl sulfide at both 8 C (46.4 F) and 30 C (86 F). Methionine, cysteine, cystathione, or β-dimethylpropioethin failed to enhance dimethyl sulfide accumulation. A cheese-whey fraction enhanced dimethyl sulfide production, as did precipitated whey protein or α-lactalbumin. Dimethyl sulfide, in this instance, is produced from sulfur-containing amino acids in peptide linkage.

CHARACTERIZATION OF TYPES OF STAPHYLOCOCCAL ENTEROTOXINS[1, 2]

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ABSTRACT

The staphylococcal enterotoxins are single polypeptide chains that contain two half-cystine residues that are cross-linked in the native toxin to form a "cystine" loop. Several amino acid residues involving part of this loop appear to be the same for the different enterotoxins which may represent the toxic site. The other part of the cystine loop may be involved in the antigenicity of the toxin which is the basis for identifying them as enterotoxns A-E. The antigenicty of the enterotoxins varies from the similarity of enterotoxins C_1 and C_2 which have the same major antigenic site to enterotoxins A and B which are apparently unrelated antigenically.

Staphylococcal enterotoxins are a closely related group of simple proteins with a molecular weight of 26,000 to 30,000 *(23)*. The enterotoxins are identified as separate entities with the letters A, B, C, etc. *(10)*, the separation being based on the major antibodies produced by the protein molecules. The specific precipitation reaction given by each enterotoxin with its own antibody is the basis for the tests used to detect the enterotoxins. Enterotoxins A *(9)*, B *(6)*, C *(3)*, D *(11)*, and E *(4)* have been identified, but how many unidentified ones exist is not known. The enterotoxins produced by many strains that are enterotoxigenic by monkey feeding tests have not been associated with specific antibodies.

TOXIC AND ANTIGENIC SITES OF THE ENTEROTOXINS

The enterotoxins are single polypeptide chains composed of relatively large amounts of lysine, aspartic and glutamic acids, and tyrosine (Table 1). They contain only one or two residues of tryptophan and only two residues of half-cystine. The latter are cross-linked to form a cystine residue as evidenced by the fact that there are no free -SH groups in the molecule *(15)*. The half-cystine residues in enterotoxin B are at positions 92 and 112 *(17)* which creates a "cystine" loop of twenty amino acid residues as shown below:

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-Gly-Ala-Asn-Tyr-Tyr-Gln-Cys-Tyr-Phe-Ser-Lys-Lys-Thr-Asn-Asn-Ile-
                          |                                  ASP
-Thr-Val-Gly-Gly-Tyr-Met-Cys-Thr-Lys-Arg-Lys-Thr-Asn-Glu-His-Ser-
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It is not known yet whether the "cystine" loop in the different enterotoxins is identical, but since only one cystine molecule is present in any of the enterotoxns it is expected that this part of the molecule is involved in some way in the activity of the toxin. Preliminary data from the sequence studies on enterotoxin A show that the six amino acid residues adjacent to the half-cystine residue at position 112 in enterotoxin B are identical. In all probability this is the active site of the molecule since the biological activity of all of the enterotoxns is the same.

The residues near the half-cystine residue at position No. 92 in enterotoxin B may comprise the major antigenic site. There are several tyrosine residues in this part of the molecule but no lysine residues are present either here or in the proposed toxic site. This is consistent with the conclusions of Spero et al. *(24)* that lysine residues are not involved in either the toxic or antigenic sites. They found that substitution of all but one of the E-amino groups of the lysine residues had no effect on the antigen-antibody reaction or the toxic action. The sequence of amino acids in this area is not the same for enterotoxin A and B which would account for these enterotoxins having different major antibodies.

One might conclude that the enterotoxins originated from a single protein and that through the course of evolution by amino acid residue substitutions, several antigenically different proteins with a common toxic site evolved. The difference in antigenicity might be used as the indicator of the degree of evolution having taken place. If a large number of enterotoxins could be purified and their sequence determined, as has been done for the cytochrome C's, one might expect to find a series of proteins that may differ only by a few amino acid residues.

ANTIGENICITY OF THE ENTEROTOXINS

Antigenically homogenous enterotoxins

There does exist within the group of enterotoxins that have been purified a rather wide range of differences in antigenicity. This range varies from those that are antigenically homogenous, but differ otherwise, to those that are distinctly different in this respect. Early work by Bergdoll showed that the enterotoxin produced by staphylococcus strain S-6

[1]Presented as a part of The Seminar on the Staphylococcal Enterotoxins, at the Annual Meeting of the American Society for Microbiology, Miami Beach, Florida, May 6-11, 1973.
[2]Contribution from the College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin.

TABLE 1. AMINO ACID COMPOSITION OF THE ENTEROTOXINS IN GRAMS PER 100 GRAMS PROTEIN

Amino acid	Enterotoxin A *(23)*	B *(5)*	C_1 *(18)*	C_2 *(18)*	E *(8)*
Lysine	11.26	14.85	14.43	13.99	10.83
Histidine	3.16	2.34	2.91	2.87	3.04
Arginine	4.02	2.69	1.71	1.75	4.50
Aspartic acid	15.53	18.13	17.85	18.38	15.10
Threonine	5.96	4.50	5.31	5.80	6.36
Serine	2.99	4.05	4.58	4.81	4.72
Glutamic acid	12.36	9.45	8.95	8.93	12.15
Proline	1.35	2.11	2.16	2.23	1.93
Glycine	2.96	1.78	2.99	2.90	4.10
Alanine	1.94	1.32	1.85	1.61	2.38
Half-cystine	0.66	0.68	0.79	0.74	0.81
Valine	4.93	5.66	6.50	5.87	4.36
Methionine	0.96	3.52	3.20	3.60	0.45
Isoleucine	4.11	3.53	4.09	4.02	4.30
Leucine	9.78	6.86	6.54	6.13	10.08
Tyrosine	10.63	11.50	9.80	10.27	9.79
Phenylalanine	4.31	6.23	5.35	5.25	4.47
Tryptophan	1.46	0.95	0.99	0.84	1.51
Amide NH_3	1.80	1.66	1.71	1.62	1.66
Total:	98.37	100.15	100.00	99.99	100.88

(later labeled enterotoxin B) could be separated into at least three fractions by careful ion exchange chromatography. All the fractions were toxic and reacted with the same specific antibody. The same type of result was reported in 1971 by Chang et al. *(13)* using hydroxyl apatite chromatography. These investigators reported that all fractions reacted with the same antibody, but that only one gave a toxic reaction in cats. Although no conclusions were drawn concerning the reasons for these differences, the ion-exchange separations would indicate the separations resulted from a difference in charge on the molecules.

Baird-Parker and Joseph *(2)* showed that purified enterotoxin B could be separated by starch gel electrophoresis into two fractions which appeared to be identical. Schantz et al. *(22)* obtained a similar separation of enterotoxin B into two fractions using starch gel. Rerunning of the slower moving material gave two bands while rerunning of the faster material gave only the one band. The material from the two bands was identical in toxicity in monkeys, in reaction wth the specific antibody, and in amino acid composition. Joseph and Baird-Parker *(20)* suggested that the difference was possibly in the secondary or tertiary structure. Schantz et al. *(22)* stated that it was uncertain whether the fractionation was due to true heterogeniety of the protein molecules or to some type of molecular interaction.

Attempts to obtain purified enterotoxin in the homogenous state by isoelectric focusing were less than successful *(12, 14)*. Enterotoxins A, B, and C purified by conventional methods gave multiple peaks when subjected to isoelectric focusing. Each peak of a given enterotoxin reacted with the specific antibody for that particular enterotoxin and appeared to be the same in every way except for the charge on the molecule. With one exception *(21)* refocusing of any given peak resulted in multiple peaks with isoelectric points equal to or lower than that of the material refocused *(12, 14)*. The conclusion one might arrive at is that the difference in charge is caused by a difference in the number of amide groups in the molecule. These results indicate that amide groups may be lost during isoelectric focusing while results from ion-exchange chromatography indicate that differences may have arisen during fermentation. These differences do not appear to be sufficiently critical to affect purification of enterotoxins when conventional methods are used.

Enterotoxins C_1 and C_2

Two enterotoxin C's have been purified and labeled C_1 and C_2, primarily on the difference in their isoelectric points, 8.6 for C_1 *(7)* and 7.0 for C_2 *(1)*. Although the major antigenic site of these two enterotoxins is identical, the observation of spur formation on Ouchterlony plates indicated a difference in their minor antigenic sites. It is possible to convert C_1 into a protein with an isoelectric point of 7.0 by removal of amide groups[3], but the antigen-antibody reactions show that the mere removal of amide groups from C_1 does not convert it to a true C_2 enterotoxin. Any differences in the amino acid composition of the two enterotoxins is within the limits of error of the analysis, hence, the difference in structure can be revealed only when the amino acid sequences of the two enterotoxins are worked out. This difference is not of practical concern since either C_1 or C_2 can be detected by the major antibody to either enterotoxin.

Enterotoxins B and C

Gruber and Wright *(16)* observed by ammonium sulfate coprecipitation that enterotoxins B and C contained similar antigenic determinant groups. During identification of enterotoxin C in the Food Research Institute no cross-reaction between the two enterotoxins was noted by antigen-antibody precipitation techniques such as the Ouchterlony gel plate *(3)*, but evidence was available which indicated that enterotoxin B antiserum did neutralize enterotoxin C. In this instance, the enterotoxin C was treated with the B antiserum before intravenous injection into rhesus monkeys. Further experiments in our laboratories indicated that enterotoxin C will no longer give a precipitate with its specific antibody after the enterotoxin is treated with enterotoxin B antiserum and vice versa. Johnson et al. *(19)* noted only a slight

[3]Private communication from Dr. Len Spero.

cross-reaction between enterotoxin B and C_1 in the solid-phase radio-immunoassay test. The amino acid composition of these two enterotoxins are similar in many respects and both have the same N-terminal amino acid. It is possible that the major antigenic sites of these two enterotoxins are sufficiently similar to give a partial cross-reaction.

Enterotoxins A and E

The enterotoxin that was produced by the staphylococcus strain chosen for use for enterotoxin E purification gave no cross-reactions with any of the antisera to the known enterotoxins. When the purification work was nearing an end, a cross-reaction between enterotoxin E and antiserum to A was noted. In this instance, antiserum to enterotoxin A neutralized enterotoxin E when injected intravenously into monkeys. Careful examination by the Ouchterlony plate test revealed that the differences were due to the major antigenic site *(4)*. The amino acid composition of these two enterotoxins are quite similar.

Enterotoxins A and B

The maximal difference in enterotoxins as antigens is between enterotoxins A and B. Although Gruber and Wright *(16)* reported some cross-reaction between these two enterotoxins, this has not been verified. No neutralization of enterotoxin B and the C's with A antiserum or vice versa has been noted, nor has any precipitate cross-reactions been observed. The amino acid composition of the two enterotoxins is different in several respects (Table 1). One of the major differences in the composition is in the methionine content, two residues for A and eight for B.

There are other differences in the enterotoxins, for example, the manner in which they are produced by the organism, but space does not permit a discussion of these differences. In any event, the antigenic differences are of greatest importance since detection methods for the enterotoxins are dependent upon their antigenic reactions.

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ROLE OF ENTEROCOCCI IN CHEDDAR CHEESE: GROWTH OF ENTEROCOCCI DURING MANUFACTURE AND CURING[1]

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ABSTRACT

Eight lots of Cheddar cheese were manufactured to determine the microbiological response of two strains each of *Streptococcus faecalis* and *Streptococcus durans* when used as supplemental starters in combination with a commercial lactic culture. Each lot consisted of a control vat of cheese manufactured with the lactic starter only, and an experimental vat of cheese containing the lactic starter and one of the enterococcus strains. Combinations of two curing temperatures (7.2 and 12.8 C) and two early cooling treatments (air vs. brine cooling) were used for cheeses from each vat to determine environmentally-induced variability.

Growth patterns were monitored throughout the manufacture period up to the end of pressing, and during curing up to 6 months. Enterococcus populations showed little or no decrease when the cheeses were being pressed, whereas populations in control cheeses decreased over the same period. During curing, control cheeses cured at 7.2 C showed marked population decreases over the 6 months; those cured at 12.8 C showed a rapid decrease followed by an upsurge in population. Populations of S. *faecalis* in the experimental cheeses decreased only slightly, and S. *durans* showed almost no decrease. Generally, cheeses cured at 7.2 C showed the greatest numerical survival and there appeared to be no population differences caused by early cooling treatment.

Any industry that relies on viable organisms for product manufacture must cope with the inherent metabolic variability of these organisms. The manufacture of Cheddar cheese is as closely controlled as practicable for a bulk fermentative procedure which is monitored, in most instances, largely by human judgment. Culture inconsistency with regard to flavor and acid production, subtle changes in temperature of manufacture and curing and differences in cool-off time caused by various stacking methods provide opportunities for cheese variability from batch to batch. These factors may have a significant effect on the metabolic activities of both starter culture organisms and adventitious flora.

An organism less susceptible to environmentally-induced variability was added to cheese milk in an attempt to achieve greater uniformity among blocks from the same batch when exposed to selected variables. The Enterococcus group of the genus *Streptococcus* was selected because of its relative durability, including heat resistance *(24)*, and because it occurs in large numbers as adventitious cheese flora *(4)*. Similar studies have been conducted by a number of other workers *(2, 3, 5-9, 11, 12, 13, 15, 17, 18, 22, 26, 27, 29)*. There is conflict among their results, and generally microbiological, chemical, and organoleptic analyses were not integrated.

It was assumed that enterococci, when used as supplemental starters, would resist change to a greater degree than normal starter organisms in number and metabolic activity with corresponding changes in the microenvironment of the cheese. To test this hypothesis, chemical, microbiological, and organoleptic analyses were done. This paper, the first of a series, discusses the microbiological aspects of the use of enterococci as supplemental starters in Cheddar cheese.

MATERIALS AND METHODS

Manufacture of cheese

Eight lots of Cheddar cheese were manufactured in the Dairy Products Laboratory, Food Technology Department, Iowa State University. Each lot consisted of a control and an experimental vat of cheese each manufactured from the same bulk milk on the same day in two 2270-kg vats. Milk used for cheese manufacture was treated by heating to 62.8 C with no hold, followed by immediate cooling. The milk was stored at 4.5 C, usually overnight, before use. Cheeses were manufactured according to the schedule and procedure outlined by Wilson and Reinbold *(28)*.

Starters used

The enterococci used as starters for cheesemaking had been previously isolated from young Cheddar cheese by Clark and Reinbold *(4)* and screened for lipolytic and proteolytic activity by Dovat et al. *(10)*. These strains were concentrated and canned by a commercial culture manufacturer. Until use, the concentrated cultures had been maintained for several months at −196 C in liquid nitrogen. Each can contained 75 ml of concentrate with viable counts of appoximately 10^{10} cells/ml. Two strains of *Streptococcus faecalis* and two of *Streptococcus durans* were used throughout the experiment.

Experimental cheese cultures consisted of 1% commercial mixed-strain Cheddar cheese starter culture in combination with 75 or 150 ml of one of the frozen enterococcus concentrates per 2270 kg of milk. The frozen concentrates were thawed quickly and carefully dispersed in a small aliquot

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of cheese milk at 30 C immediately before addition to the vat. Control cheeses were made using 1% of the same commercial mixed-strain lactic culture for all eight lots. Lot designations are given in Table 1.

Treatment of cheese

Forty-pound blocks were pressed overnight (20 h) at approximately 21 C and then wrapped in Marathon foil-cellofoil wrappers (Marathon, Division of American Can Company, Neenah, Wisc.) and sealed with a Flexpress model R.L. 100 (D. L. Manufacturing Company, De Pere, Wisc.). Half of the blocks from each vat (control and experimental) were then cooled rapidly by immersion in 7.5 C brine for 5 days *(19, 20)*, and the other half were immediately placed in curing rooms to air cool. From each of these early cooling treatments, half of the blocks were cured at 7.2 C and the other half at 12.8 C.

Sampling

Samples taken during the manufacturing period were held at 10 C in sterile Whirl-Pak (Nasco Company, Fort Atkinson, Wisc.) containers for bacteriological analysis at the earliest convenient time (always within 2 h). Samples of curing cheese were taken aseptically and plated immediately. For bacterial counts, samples were taken during the manufacture period after starter addition, after rennet addition, after cook, after milling, and after pressing for 20 h. During the curing period, samples were collected at 5, 10, 30, 60, and 90 days and at 6 months.

Micobiological analysis

Total counts and enterococcus counts were made on all samples. The cheeses were prepared for plating as specified in *Standard Methods for the Examination of Dairy Products (1)*. For total bacterial counts, the proper dilution series was plated in duplicate in Eugonagar (Baltimore Biological Laboratories, Baltimore, Md.) and incubated at 32 C for 7 days. For enumeration of enterococci, similar dilutions of enterococcus cheeses and their corresponding controls were plated in Eugonagar and incubated at 45 C for 3 days. When control cheeses were plated at 45 C, there were no colonies evident at the same dilutions used to enumerate enterococci in the experimental cheeses. Consequently, it was assumed that colonies growing at 45 C resulted from the deliberate addition of enterococci.

RESULTS

Growth during manufacture

All eight lots exhibited similar growth and survival patterns during the make period. Figure 1 shows trends in total bacterial counts for each negative control cheese to the end of the pressing period. The heavy line is a composite representation of the overall response. In all instances, the greatest increase in numbers occurred between the addition of the rennet and the end of the cook period. After reaching their maximum, usually following cooking, numbers declined steadily to the end of the press period. The degree of population decline between milling and the end of the press period for all lots was from 35% to 94%, with an average decline of 73%.

Figure 2 shows growth and survival patterns of enterococci in the eight lots of cheeses made with a supplemental enterococcus starter in combination with

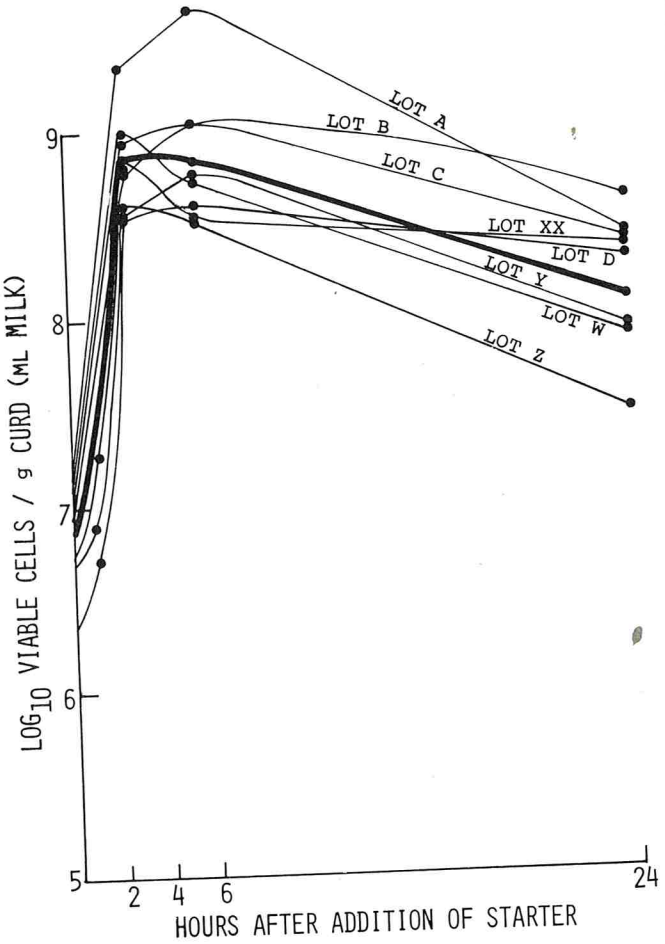

Figure 1. Total bacterial counts of control cheeses during manufacture.

a commercial lactic starter. The increase in population during the ripening period was not as great as that of the lactic streptococci in the negative control vats. In all lots, the maximum population of enterococci was reached at milling, and with the exception of Lot A, this level was either maintained or increased slightly until the end of the press. Two lots (C and W) decreased by 13.3% and 14.2%, respectively, in this period, and in the other six lots, enterococcus populations increased from 3.6% to 36.3%.

Total bacterial counts in cheeses made with enterococci (representing numbers of both lactic starters and enterococci) exhibited much the same pattern as the total counts in the control cheeses, with the exception of a less pronounced decline in numbers after milling.

Growth during curing

Survival patterns during curing at 7.2 C for all eight lots of control cheese are given in Figure 3. For simplicity, only the air-cooled cheeses are represented in the figure because the brine-cooled cheeses followed a nearly identical pattern. The heavy line is a composite representation of the overall response.

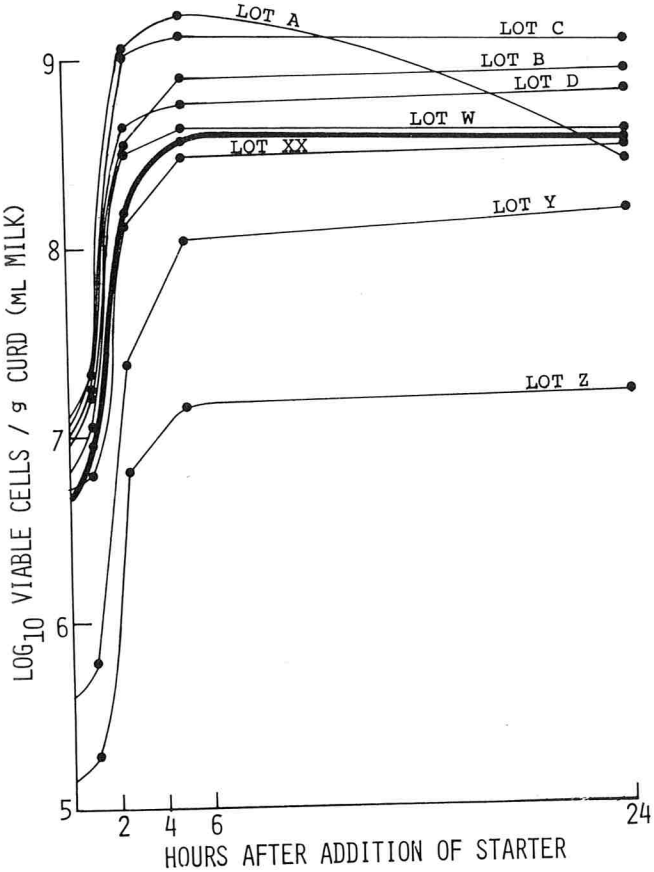

Figure 2. Enterococcus counts of experimental cheeses during manufacture.

There was a rapid decline in numbers initially, especially during the first 5 days of curing. Although some cheeses showed total bacterial count increases between 10 and 30 days, the population continued to decrease gradually. In all instances, the total population of the control cheeses decreased as much as 3.5-4.0 log units. Lots W and XX, however, increased in count after 90 days of curing.

The curing-period growth response of control-cheese flora, air-cooled and cured at 12.8 C is presented in Figure 4. As with cheeses cured at 7.2 C, there was an initial rapid decline in population. Starting from 10 days to as long as 90 days, however, this decline was followed by a rather abrupt increase in numbers. Although the general trend of the eight lots is the same, again there were wide variations in the size of population and in the stage at which the increase occurred.

Figure 5 shows the growth of enterococci during curing in the four lots of cheese made with S. *faecalis* as a supplemental starter. Data presented includes cheeses, air-cooled, cured at both 7.2 C and 12.8 C. There was a gradual decrease in enterococcus count over the 180-day period, with the smallest decrease of 0.7 log units occurring in Lot B when cured at 7.2 C. The greatest decrease of about 1.3 log units was observed in Lot D cured at 12.8 C. When compared with the total counts in control cheeses, it is obvious that S. *faecalis* survives the environment of curing cheese better than normal starter lactic streptococci. Survival at 12.8 C, however, is not quite as great as at 7.2 C.

Enterococcus counts in cheeses made with S. *durans* as the supplemental starter are given in Figure 6. The population decrease was markedly less than that exhibited by S. *faecalis*. Up to the 90-day sampling period, counts in all instances maintained essentially constant numbers. Between 3 and 6 months, there was a slight numerical decrease except in Lot Y at 12.8 C and Lot XX at 7.2 C. In Lots W and XX, which were manufactured with 150 and 75 ml of S. *durans* 15-20 concentrate, respectively, curing temperatures influenced the rate of survival; cheeses cured at 12.8 C had somewhat lower counts than those at 7.2 C after 180 days. The two lots made with S. *durans* 9-20 (Lots Y and Z) exhibited the reverse trends.

When an analysis of variance was done on the enterococci populations in the experimental cheeses, only a slight significant difference in population was

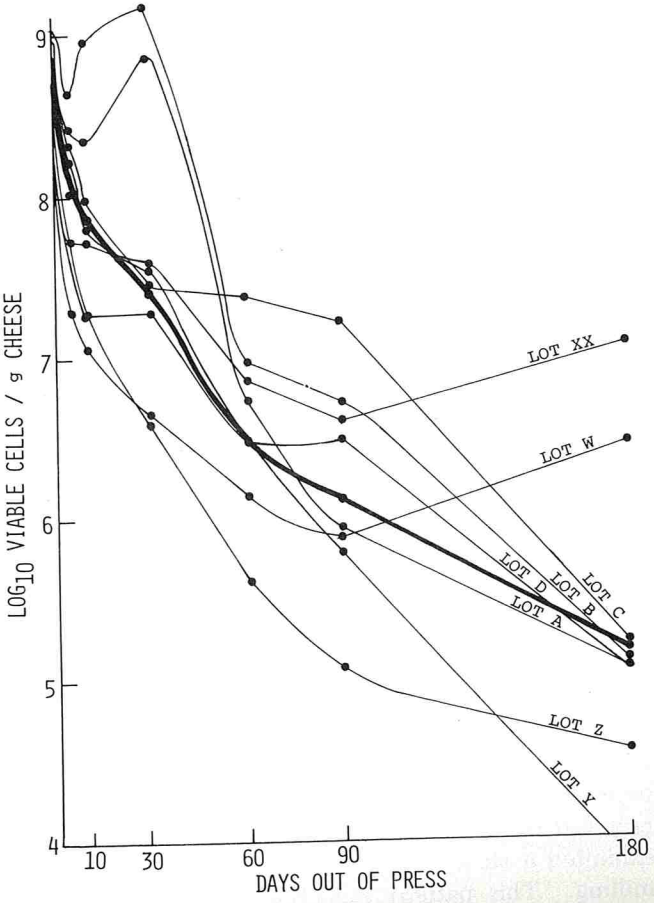

Figure 3. Total bacterial counts of air-cooled control cheeses cured at 7.2 C.

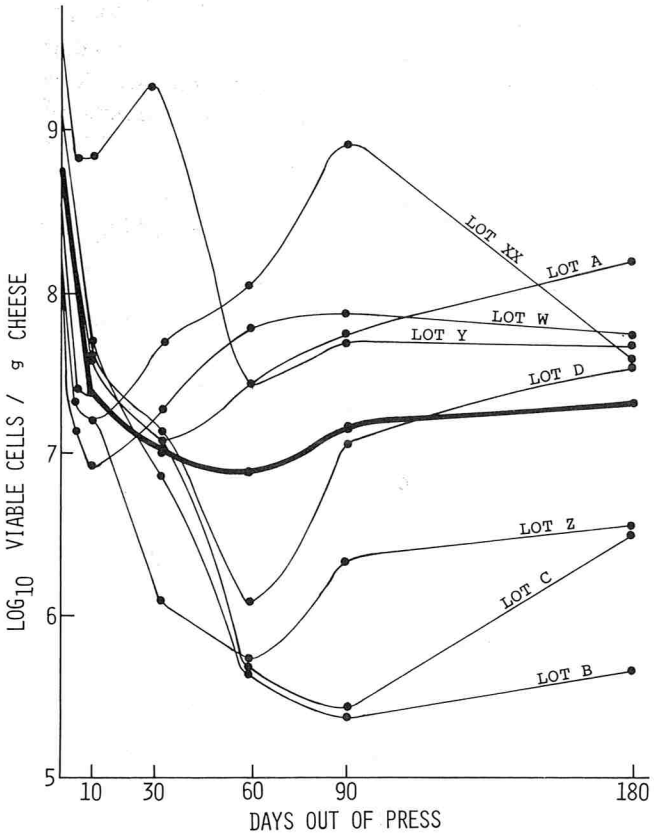

Figure 4. Total bacterial counts of air-cooled control cheeses cured at 12.8 C.

observed when the cheeses were subjected to different curing temperatures. The total counts in the control cheeses exhibited significant differences to much greater degree when the curing temperature was varied. The differences in populations in days of cure (each sampling point) are statistically significant for both control and experimental cheeses, although to a much greater degree for the control cheeses. In control cheeses, the interaction between curing temperature and days of cure is significant whereas it is not in the experimental (enterococci) cheeses. Statistical results are presented in Table 2.

Discussion

Although the supplemental enterococci in the experimental lots did not multiply as rapidly as the starter in the control group during the initial stages of manufacture, the enterococci either maintained constant numbers or slightly increased in number between milling time and the time the cheese was removed from the press. The control starter cheeses exhibited a steady bacterial numerical decrease after milling. This pattern probably resulted because of the capacity of enterococci to tolerate the acidity and brine-salt concentration of Cheddar cheese. Such survival may be desirable since, with a large number of actively metabolizing enterococci, the available carbohydrate will be more quickly utilized than in the control cheeses where the majority of organisms are in a numerically declining phase. This enterococcal survival has a two-fold advantage; first, more rapid depletion of lactose would decrease the likelihood of growth of undesirable microorganisms, and second, more complete fermentation of the carbohydrate would insure the stabilization of the pH of the finished cheese in the desirable range of 5.1-5.3 during curing. Changes of this nature will be reported in a future paper.

Survival patterns during curing of the eight negative control lots are generally similar for each curing temperature, but population variances are evident among the lots. These differences are likely due to the presence in the cheese milk of different types and proportions of adventitious flora (different growth responses under the curing conditions). We did not verify this occurrence but Feagan and Dawson *(14)* have observed that individual cheeses of similar age and from similar milk source showed considerable variability in the types of microorganisms present. Thus, that each control lot would behave identically would be unexpected.

The increase in population in normal starter (nega-

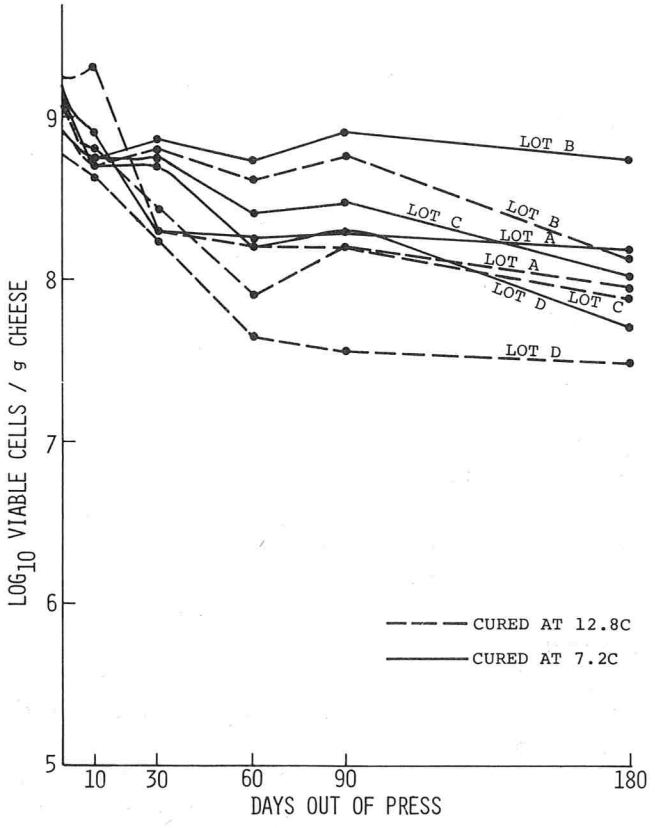

Figure 5. Enterococcus counts during cuing of air-cooled experimental (*S. faecalis*) cheeses.

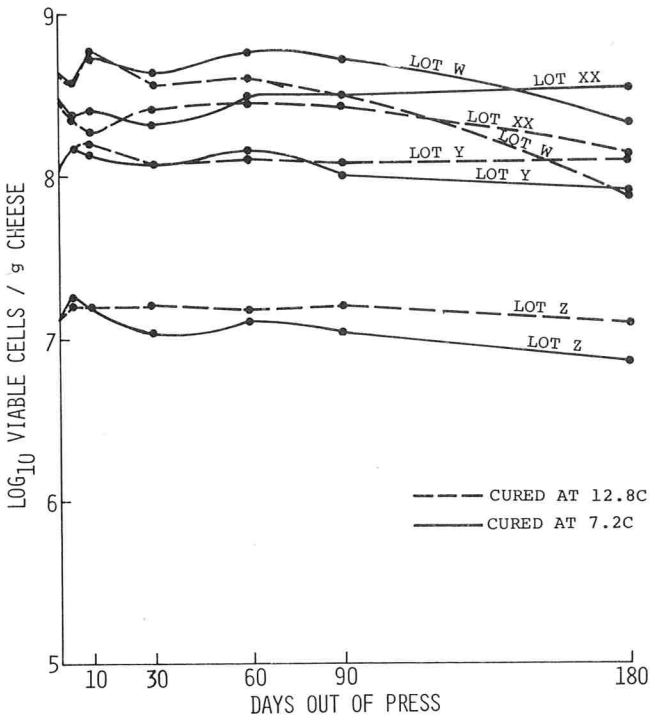

Figure 6. Enterococcus counts during curing of air-cooled experimental (*S. durans*) cheeses.

tive control) cheeses when cured at 12.8 C was observed as long as 78 years ago by Russell *(23)*, who reported that the numbers of bacteria in fresh curd decline for a day or so, followed by an increase. At the time that report was made the accepted curing temperature was rather high ($>$ 12.8 C) and thus compares with the present results. Several workers have indicated that lactobacilli predominate after the starter organisms have died. Feagan and Dawson *(14)* stated that lactobacilli constitute 80% of the non-starter population, and Johns and Cole *(16)* claimed that lactobacilli multiplied rapidly even during the first 1 or 2 days of curing, reaching their maximum numbers in 3 to 6 months. These reports indicate that the increase in population in negative control cheeses cured at 12.8 C may be due to a proliferation of lactobacilli at this temperature.

The high survival rate of *S. faecalis* in curing Cheddar cheese as observed in this study also was noted by Kosikowsky and Dahlberg *(17)*. That *S. faecalis* is able to survive well in curing cheese is probably due to its salt tolerance, acid tolerance, and general resistance to the microenvironment of the cheese. The more rapid decline in population at 12.8 C may result from a more rapid metabolism as compared with activity at 7.2 C. More rapid metabolism would cause a faster depletion of fermentable carbohydrate in the microenvironment. A corollary to this would be the more rapid accumulation of autotoxic end-products resulting in a more rapid rate of cellular death.

The maintenance of an almost unaltered population of *S. durans* in curing cheese leads to speculation on the probably unique nature of the microorganism. It obviously possesses properties that *S. faecalis* does not and that enable sustained survival in the microenvironment of the cheese. Perhaps it metabolizes more slowly than *S. faecalis* almost to the point of dormancy, which would prolong the supply of nutrients and avoid rapid accumulation of toxic by-products. Such behavior, however, would make the presence of *S. durans* unlikely to have much effect on cheese flavor. A second possibility is that *S. durans* accumulates different and less toxic by-products than does *S. faecalis*, which could affect cheese flavor. But such an explanation still leaves the question of depletion of nutrients. There is the possibility that *S. durans* is able to use other components as an energy source, for example citrate, after preferential utilization of lactose, glucose, and galactose. It is well known, however, *(21, 25)* that *S. faecalis* has a broader

TABLE 1. EXPLANATION OF EXPERIMENTAL VARIATION BETWEEN CHEESE LOTS[a, b]

Lot	Species and strain of enterococcus	Amount of concentrate added/2270 kg milk
A	*S. faecalis* 47-13	150 ml
B	*S. faecalis* 47-13	75 ml
C	*S. faecalis* 24-23	150 ml
D	*S. faecalis* 24-23	75 ml
W	*S. durans* 15-20	150 ml
XX	*S. durans* 15-20	75 ml
Y	*S. durans* 9-20	150 ml
Z	*S. durans* 9-20	75 ml

[a]All experimental cheeses were made with 1% commercial mixed-strain lactic starter in addition to enterococcal inoculation.

[b]All control cheeses were manufactured with 1% commercial mixed-strain lactic starter.

TABLE 2. ANALYSIS OF VARIANCE; CONTROL CHEESE POPULATIONS VS. ENTEROCOCCUS POPULATIONS IN EXPERIMENTAL CHEESES[a]

Source of variation	d.f.	F values	
		Control	Experimental
Curing temperature (T)	1	14.66**	5.66*
Cooling procedure (C)	1	0.08	0.25
C × T	1	0.29	0.03
Days of curing (D)	6	65.50**	25.36**
C × D	6	0.19	0.25
T × D	6	13.40**	1.14
C × T × D	6	0.05	0.14
Error[b]	189	0.433	0.071

[a]All cheeses were manufactured with 1% commercial mixed-strain lactic starter. Experimental cheeses contained enterococcus starters as specified in Table 1.

[b]Mean square error term given.

*Significant at $P < 0.05$.

**Significant at $P < 0.01$.

fermentation spectrum than does S. *durans*, and typical S. *faecalis* would be expected to be more versatile in scavenging energy sources. Other possible explanations are that S. *durans* is more salt and acid tolerant, possibly grows anaerobically more easily, and has a metabolic pattern and rate conducive to maintaining a suitable microenvironment longer. Indeed, since certain workers *(21, 25)* claim that S. *faecalis* has more fermentative and reductive powers than S. *durans*, it is likely that S. *faecalis* must produce a greater amount of energy for its more complex systems to operate, thus quickly using the resources of the cheese microenvironment.

Statistical results indicate considerably more population uniformity in enterococcus cheeses regardless of treatment variability as compared with the populations in the negative control cheeses. This indicates that the survival of enterococci when used as a starter is less susceptible to environmental changes, and the overall quality of these cheeses may be more predictable from batch to batch if chemical changes exhibit the same uniformity. Survival of enterococci also may serve to salvage cheese which has lost lactic-starter activity due to bacteriophage infection.

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TREATMENT OF CONCENTRATED PIMIENTO WASTES WITH POLYMERIC FLOCCULATING AGENTS

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Abstract

Chemical methods of treatment were investigated as a means to reduce the waste load of concentrated unit effluents from pimiento canning. Polymeric flocculating agents and inorganic salts were applied to unit effluents. Optimum laboratory conditions for suspended solids removal were then tested on a pilot plant scale. Turbidity of effluents from peel removal, core removal, and the total canning operation composite were reduced by 95, 93, and 74%, respectively. Corresponding values for suspended solids reduction were 95, 82, and 86%. Segregation and separate treatment of the peel removal effluent which contains 69% of the total load of suspended solids in only 18% of the waste water flow is recommended.

Many food processing industries are being required to adopt complex and costly provisions for handling of waste effluents. Difficulties are confounded by a number of factors, especially wide variations in volume and composition of the effluents on a seasonal and day-to-day basis. This is particularly true for fruit and vegetable canning operations where effluents from each commodity and each unit operation are unique in character whether alone or in combination. The variability and frequent high concentration of these effluents place severe limitations on the application of standard biological waste treatment practices. Reduction of specific effluent wastes by physical-chemical reactions such as coagulation of suspended solids and carbon adsorbtion of dissolved solids may offer more effective treatment. Chemical treatment with flocculating agents may be especially useful as a means to reduce the suspended solids load to meet pre-treatment discharge standards which are being developed by the Environmental Protection Agency.

Several workers have reported the wide variability of waste loads in canning effluents of different commodities and unit processes. Mercer et al. *(8)* have characterized the variations of in-plant waste streams from the canning of peaches and tomatoes. Splittstoesser and Downing *(13)* reported waste effluent data for factories processing apples, beets, carrots, cherries, corn, green beans, grape juice, peas, pumpkin, sauerkraut, and tomatoes. Shewfelt and Chipley *(12)* reported wide differences in the character of effluents from unit processes of a dry bean canning operation. In a subsequent report by Shewfelt *(11)*, preliminary data were given on the use of polymetric flocculants and granular activated charcoal for the reduction of waste load in the processing effluents of leafy greens, pimientos, white potatoes, and poultry by-products.

Pimientos are canned mostly in California and in Georgia. It is estimated that more than 18,000 tons of pimientos were canned in 1972. The crop is of considerable economic importance because it provides income for a relatively large number of small-acreage growers and also for a significantly large number of cannery workers. Large volumes of water required for pimiento processing cause excessive dilution of the composite waste effluent and suggest consideration of separate physical-chemical treatment of certain unit process effluents *(3)*. The present study was concerned with the application of chemical flocculation treatments to the waste effluents from commercially-canned pimientos.

Physical-chemical treatment techniques including flocculation and adsorption, have been successfully applied to municipal wastes *(1, 2)*. These techniques are also applicable to food processing wastes which are often much more concentrated than municipal wastes and are often more variable due to processing schedules. Hopwood and Rosen *(5)* reduced the pollution load of concentrated slaughterhouse and poultry packing wastes by treatment with sodium lignosulfonate. A review by the National Canners Association *(9)* of liquid processing wastes indicated that inorganic salts such as lime, alum, or iron sulfates were the most common coagulating agents applied to liquid vegetable wastes. The efficiency of removal of suspended material by coagulation and settling ranged from 50 to 90% for pea, beet, corn, bean, tomato, and carrot wastes. No studies involving polymeric flocculating agents were reported.

A mechanism of action of polymeric flocculating agents is described by LaMer and Healy *(6)* in which the polymer destabilizes a collodial suspension by adsorption of particles and subsequent formation of particle-polymer-particle bridges. This is generally true for anionic and nonionic polyelectrolytes which are used to coagulate negative colloids. Cationic

TABLE 1. LABORATORY STUDY OF THE EFFECT OF POLYMER CONCENTRATION ON SUSPENDED SOLIDS REMOVAL FROM PIMIENTO PEEL REMOVAL EFFLUENT AT pH 4.0[1]

Natron 86, mg/l	Suspended solids in settled supernatant, mg/l
0	45
5	49
10	56
20	52
30	52
50	16
70	82
100	116

[1]The concentration of suspended solids in the raw waste was 230 mg/l.

polymers which are positively charged can destabilize a negative collodial suspension by charge neutralization as well as bridge formation according to O'Melia *(10)*.

The objective of this study was to investigate the effects of treatment with polymeric flocculating agents on reduction of waste loads of pimiento canning effluents with particular emphasis on in-plant streams of relatively high concentration.

MATERIALS AND METHODS

Effluent samples

Samples of concentrated processing effluents were collected from a large-scale pimiento canning operation at the following plant locations: peel removal operation, effluent A; core removal operation, effluent B; citric acid dip, packing, and closing area, effluent E; and the sewer line carrying the total plant composite effluent, effluent F *(3)*. All samples were screened at the plant through a 20-mesh screen. Each sample consisted of a 10 to 20-gal portion which was stored at 3 C within 15 min of collection and treated within 2 days. No significant changes in the concentration of suspended solids or COD were observed during this storage period.

Effluents C and D from the hand grading and cleaning areas were not tested in these treatment studies due to their dilute nature *(3)*.

Laboratory studies

The effects of various combinations and concentrations of flocculating agents on reduction of waste load of the four effluents were investigated on a laboratory scale. Of eight polymers tested, two cationic polymers (Natron 86 and WT-2660) and one anionic polymer (WT-3000) were particularly effective in removing suspended solids from pimiento waste effluents. These polymers were tested separately and in combination with each other and with inorganic salts including ferric chloride ($FeCl_3$) and alum ($Al_2(SO_4)_3$). Effectiveness of polymer treatments was evaluated over a polymer concentration range of 0 to 100 mg/1. The effect of pH on flocculation treatments was also examined.

A laboratory stirrer with four stirring paddles (2.6 × 8.6 cm) and rotating at a variable controlled speed was used to mix 500-ml volumes of waste effluent contained in 800-ml beakers. Upon addition of the desired chemical agents, the contents of each beaker were mixed at 125 rpm for 4 min and then allowed to settle for 60 min. These conditions resulted in lower suspended solids and turbidity values in the supernatant than when chemicals were mixed at 100-200 rpm for 1 min and at 30 rpm for 4-5 min followed by settling. The supernatant liquid was decanted with care to prevent disturbing the settled materials and analyzed for suspended solids *(7)* and turbidity *(4)*. A Hach Laboratory Turbidimeter, Model 2100A, was used for turbidity measurements.

Those sets of conditions in laboratory studies which resulted in greatest reductions in the concentration of suspended solids and turbidity were selected for further study on a pilot-plant scale.

Pilot-scale studies

The pilot-scale unit illustrated in Fig. 1 was employed for the larger scale flocculation studies. While the charcoal adsoption column of the unit was used with selected effluent samples, the limited data obtained were not included in this paper. The top mixing tank was charged with 37.85 liters (10 gal) of waste effluent. A variable-speed stirring paddle was rotated in this tank at approximately 125 rpm. The chemical flocculating agents contained in 500 ml water were added to the mixing tank at a rate of 25 ml/min. Concentrations of polymer and salt added were those determined to be optimum in laboratory studies on the different unit effluents. The mixture was then transferred by gravity to the settling tank and allowed to settle while being stirred slowly with a rotating baffle at 6 rpm. Samples were decanted

TABLE 2. PILOT-SCALE STUDIES OF THE EFFECTS OF CHEMICAL TREATMENTS ON THE TURBIDITY, SUSPENDED SOLIDS, AND COD OF PIMIENTO ROASTER WASHER (PEEL REMOVAL) EFFLUENTS

Treatment no.	Chemicals added (final concn.)	pH	Samples taken	Time (h)	Turbidity, FTU	Suspended solids, mg/l		COD, mg/l	
					Trial 1	Trial 1	Trial 2	Trial 1	Trial 2
1	Natron 86 (50 mg/l) Alum (40 mg/l)	4.0	Raw	0	20	230	214	1940	1882
			Decanted	0.5	1.1	17	1	—	1419
			Decanted	1	1.0	11	1	1533	1419
			Decanted	2			2		1407
2	Natron 86 (5 mg/l) WT-3000 (5 mg/1) Alum (40 mg/l)	4.0	Raw	0	—[a]	209		2009	
			Decanted	2	—	33		1581	
3	Natron 86 (25 mg/l) Alum (40 mg/l)	4.0	Raw	0	—	221		—	
			Decanted	0.5	—	112		—	
			Decanted	1	—	85		—	

[a]Not determined

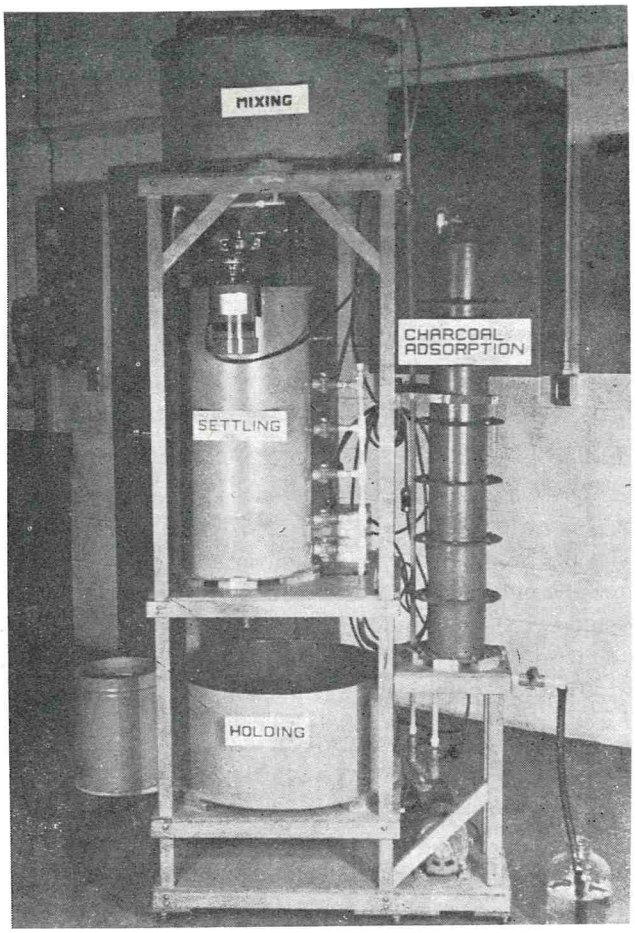

Figure 1. A pilot-scale unit for physical-chemical treatment studies.

every 30 min from the lowest sampling port on the side of the settling tank.

Since suspended solids accounted for most of the turbidity of these wastewaters, the assay of turbidity was used to monitor the course of settling of flocculated solids. The concentrations of suspended solids and COD in the samples taken were determined later. Since both soluble and suspended solids contribute to the COD, no attempt was made to correlate COD with turbidity.

The pilot-scale treatments applied were as follows:

Effluent	Treatment no.	pH	Chemical agents
Peel removal	1	4.0	Natron 86 (50 mg/l), alum (40 mg/l)
	2	4.0	Natron 86 (5 mg/l), WT-3000 (5 mg/l), alum (40 mg/l)
	3	4.0	Natron 86 (25 mg/l), alum (40 mg/l)
Composite	1	4.6	Natron 86 (30 mg/l), $FeCl_3$ (40 mg/l)
	2	5.1	WT-2660 (2 mg/l)
Core removal	1	6.0	Natron 86 (60 mg/l), alum (80 mg/l)
	2	6.1	Natron 86 (60 mg/l), $FeCl_3$ (10 mg/l)
Citric acid dip	1	3.8	None

RESULTS AND DISCUSSION

Effluent from peel removal operation

Removal of the waxy peel from pimientos by roasting resulted in a considerable amount of charred material which was subsequently washed into the peel removal effluent. The suspended solids load of this unit effluent was 2.2 lb per ton of raw material processed *(3)*. This accounted for 69% of the total load of suspended solids from all processing operations yet was contained on only 18% of the waste volume. Thus, segregation and separate treatment of this concentrated effluent would significantly reduce the total load of suspended solids.

The natural pH of the peel removal effluent was 6.0 $\pm$ 0.2 *(3)*. In laboratory studies, lowering the pH to 4.0 followed by stirring for 4 min and settling for 60 min resulted in a 44% decrease in the suspended solids concentration over that of the raw settled effluent. The effect of varying the polymer concentration to determine the optimum amount for effective treatment (Table 1) indicated that the lowest suspended solids concentration in the settled supernatant was obtained with a polymer concentration of 50 mg/l. Further increases in the polymer concentration caused restabilization of the flocculated solids due to overdosing *(10)*. Treatment No. 1 of Table 2 shows the results obtained from pilot-scale treatment of the pimiento peeling effluent.

As shown in Table 2, chemical treatment (No. 1, trial 1) with Natron 86 (50 mg/l) and alum (40 mg/l) at pH 4.0 followed by primary settling resulted in a 95% reduction in the turbidity from 20 to 1.0 Formazin Turbidity Units (FTU) and a 95% reduction in the suspended solids concentration, from 230 to approximately 11 mg/l. Removal of this suspended material accounted for a reduction of approximately 21% in the COD of the decanted supernatant from 1940 to 1533 mg/l. The values shown under trial 2 are from a replicate of Treatment No. 1 applied to a different batch of effluent.

Treatment No's. 2 and 3 applied to the peel removal effluent proved to be less effective than the conditions employed in Treatment No. 1. The combination of a cationic polymer, Natron 86, and an anionic polymer, WT-3000, in Treatment No. 2 resulted in an 84% reduction in the suspended solids concentration from 209 to 33 mg/l. Treatment No. 3 which employed half the concentration of Natron 86 (25 mg/l) as compared to Treatment No. 1, resulted in a 62% reduction in the concentration of suspended solids from 221 to 85 mg/l.

Composite effluent

The combined unit effluents resulted in a composite effluent which contained 3.2 lb. suspended

TABLE 3. LABORATORY STUDY OF THE EFFECT OF POLYMER AND FERRIC CHLORIDE CONCENTRATIONS ON SUSPENDED SOLIDS REMOVAL FROM THE COMPOSITE PIMIENTO EFFLUENT AT PH 4.6[a]

Natron 86, mg/l	pH	$FeCl_3$, mg/l	Suspended solids in settled supernatant, mg/l
10	4.6	0	40
10	4.6	40	27
30	4.6	0	44
30	4.6	40	17
50	4.6	0	53
50	4.6	40	36
0	4.6	40	42
30	6.0	40	57
30	8.0	40	78

[a]The concentration of suspended solids in the raw waste was 100 mg/l.

solids/ton and 60.2 lb. COD/ton of raw material processed. The total flow of 849 gal/min corresponded to 4,840 gal waste water per ton *(3)*. Treatment of this relatively dilute effluent with different concentrations of flocculant showed that 30 mg/l Natron 86 and 40 mg/l $FeCl_3$ (Table 3) resulted in reduction of the suspended solids concentration by approximately 83%. Without polymer treatment, a reduction of approximately 58% was obtained by settling.

Results of chemical treatment of the composite effluent on a pilot-scale with Natron 86 (30 mg/l) and $FeCl_3$ (40 mg/l) at the natural pH of raw composite effluent (4.6-5.1) are shown in Table 4. Flocculation of suspended materials resulted in a 74% reduction in the turbidity from 17 to 4.4 FTU, and an 86% reduction in the concentration of suspended solids from 100 to 14 mg/l. Removal of this material accounted for a reduction of approximately 19% in the COD of the decanted supernatant from 970 to 790 mg/l. Replicate values are shown under trial 2.

Treatment No. 2 with WT-2660 (2 mg/l) resulted in little decrease in the turbidity, but effected a 57% decrease in the concentration of suspended solids from 67 to 29 mg/l.

Effluent from core removal operation

The core removal operation produced an effluent which contained a high proportion of soluble organic components as shown by a COD load of 13.8 lb./ton and a much lower suspended solids load of 0.4 lb./ton *(3)*. Laboratory-scale studies on this effluent (Tables 5 and 6) indicated that treatment with 60 mg/l of the cationic polymer Natron 86 plus either 80 mg/l alum or 10 mg/l ferric chloride effectively reduced the turbidity and suspended solids concentration.

The results of pilot-scale chemical treatments on the core removal effluent are shown in Table 7. Treatment No. 1 with Natron 86 (60 mg/l) and alum (80 mg/l) at the natural pH of the raw unit effluent (6.0) caused a 93% reduction in turbidity, from 17 to 1.2 FTU. In addition, the concentration of suspended solids was reduced by 82%, from 76 to 14 mg/l. Removal of this suspended material accounted for a reduction in the COD of only 8.4%. Replicate values for suspended solids and COD are shown under trial 2.

The conditions of Treatment No. 2, Natron 86 (60 mg/l) and $FeCl_3$ (10 mg/l) at the natural pH of the raw effluent, were almost as effective as those of Treatment No. 1. The turbidity was reduced from 18 to 2.2 FTU, the suspended solids from 36 to 16 mg/l, and the COD from 1510 to 1469 mg/l.

Effluent from the citric acid dip, packing, and closing area

The suspended and dissolved solids concentrations of this effluent were 34 and 5,057 mg/l, respectively. The pH was 3.8 due to the citric acid dip. None of the chemical agents nor the different pH conditions tested were as effective as simply allowing the effluent to coagulate and settle for 1 h without further addition of chemicals. This simple procedure reduced the suspended solids concentration by 91% from 34 to 3 mg/l.

CONCLUSIONS

Advantages of physical-chemical treatment

Chemical flocculation of concentrated pimiento effluents was effective in reducing the turbidity and suspended solids when the appropriate conditions were determined. These techniques should be widely applicable in the food processing industry for reducing the waste load and treatment charges due to concentrated unit effluents. Also, by-products may be developed from the flocculated solids.

The major anticipated benefits of flocculation and other physical-chemical treatment systems to the food processor are: *(a)* ease of start-up and shut-down; *(b)* flexibility to handle shock loads; *(c)* specific treatment for different products; *(d)* small land area; *(e)* design flexibility for desired treatment efficiency; and *(f)* possibility for recovery of by-products from chemical treatment stage.

Separate treatment of concentrated effluents

The determination of optimum conditions and the effectiveness of chemical flocculation is a function of the chemical composition, pH, and concentration of the different effluents. The concentrated effluent from the peel removal operation which contained over 200 mg/l suspended solids was treated to remove 95% of the suspended solids and turbidity by a combination of Natron 86 cationic polymer (50 mg/l) and alum (40 mg/l) at a pH of 4.0. The effluent from the core removal operation which contained 76 mg/l

TABLE 4. PILOT-SCALE STUDIES OF THE EFFECTS OF CHEMICAL TREATMENTS ON THE TURBIDITY, SUSPENDED SOLIDS, AND COD OF COMPOSITE PIMIENTO EFFLUENTS

Treatment no.	Chemicals added (final concn.)	pH	Samples taken	Time (h)	Turbidity, FTU	Suspended solids, mg/l		COD, mg/l	
					Trial 1	Trial 1	Trial 2	Trial 1	Trial 2
1	Natron 86 (30 mg/l)	4.6	Raw	0	17	100	100	970	882
	$FeCl_3$ (40 mg/l)		Decanted	0.5	6.5	31	32	—	—
			Decanted	1	3.9	16	14	—	—
			Decanted	1.5	3.8	12	10	—	—
			Decanted	2	4.4	14	14	790	686
2	WT-2660 (2 mg/l)	5.1	Raw	0	18	67		972	
			Decanted	0.5	18	37		—	
			Decanted	1.0	18	—[a]		—	
			Decanted	1.5	17	—		—	
			Decanted	2.0	17.5	30		—	
			Decanted	2.75	17	29		744	

[a]Not determined

suspended solids was treated optimally with Natron 86 (60 mg/l) and alum (80 mg/l) at pH 6.0 which removed 82% of the suspended solids and 93% of the turbidity. Thus, larger amounts of polymer and alum were required to treat the effluent having the lower concentration of suspended solids. Chemical flocculation with polymers was completely ineffective in the case of the effluent from the citric acid dip, packing, and closing area which contained only 34 mg/l suspended solids.

The concentration of suspended solids in the composite effluent was 100 mg/l which was sufficiently concentrated to respond to chemical flocculation. Treatment with Natron 86 (30 mg/l) and $FeCl_3$ (40 mg/l) at pH 4.6 reduced the suspended solids by 86% and the turbidity by 74%.

These results show that chemical flocculation could be used to treat the total composite waste from a pimiento canning operation, but a preferred approach is suggested by the results obtained on separate treatment of concentrated effluents from unit operations. For example, the peel removal operation has been shown to contribute 69% of the load of suspended solids from the total pimiento canning operation, but this effluent accounts for only 18% of the total flow of waste water *(3)*. This suggests that a more efficient means of reducing the pollution load of this effluent would be to treat it while still a concentrated stream, rather than after it has been diluted with weaker wash waters. The experimental results obtained in this study are in agreement with this hypothesis and suggest that segregation and separate treatment of concentrated unit effluents should be considered as a viable option in the planning of pollution control procedures and facilities.

ACKNOWLEDGMENTS

The polymeric flocculating agents WT-3000 and WT-2660 were supplied by Calgon Corporation; Natron 86 was supplied by National Starch and Chemical Corporation. The technical assistance of Susan Nolan and Stan Donehoo is gratefully acknowledged. The contributions of Mr. Gordon Futral and the staff of the Agricultural Engineering Department and Mr. Ralph Koons of the Food Science Department of the Georgia Experiment Station in the design and construction of the pilot-scale treatment unit are acknowledged.

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TABLE 5. LABORATORY-SCALE TREATMENT OF PIMIENTO CORE REMOVAL EFFLUENT WITH VARYING CONCENTRATIONS OF POLYMER AND ALUM AT PH 6.0

Natron 86, mg/l	Alum mg/l	Suspended solids, mg/l	Turbidity, FTU
50	0	—	15
50	10	—	3.4
50	40	22	2.8
50	80	18	2.3
50	120	12	2.2
0	0	—	14
40	80	—	3.7
50	80	—	3.3
60	80	20	3.1
70	80	—	3.1

TABLE 6. LABORARORY-SCALE TREATMENT OF PIMIENTO CORE REMOVAL EFFLUENT WITH VARYING CONCENTRATIONS OF POLYMER AND FERRIC CHLORIDE AT PH 6.0

Natron 86, mg/l	$FeCl_3$ mg/l	Suspended solids, mg/l	Turbidity, FTU
50	0	26	4.7
50	4	28	4.2
50	10	20	3.7
50	20	26	4.1
50	30	24	5.6
50	40	45	17
0	0	—	14
40	10	—	4.2
50	10	—	4.0
60	10	—	3.7
70	10	—	4.2

TABLE 7. PILOT-SCALE STUDIES OF THE EFFECTS OF CHEMICAL TREATMENTS ON THE TURBIDITY, SUSPENDED SOLIDS, AND COD OF EFFLUENTS FROM A PIMIENTO CORE REMOVAL OPERATION

Treatment no.	Chemicals added (final concn.)	pH	Samples taken	Time (h)	Turbidity, FTU	Suspended solids, mg/l		COD, mg/l	
					Trial 1	Trial 1	Trial 2	Trial 1	Trial 2
1	Natron 86 (60 mg/l)	6.0	Raw	0	17	76	44	1615	1298
	Alum (80 mg/l)		Decanted	0.5	1.2	14	16	1498	—[a]
			Decanted	1	1.2	14	16	1479	1232
2	Natron 86 (60 mg/l)	6.1	Raw	0	18	39		1510	
	$FeCl_3$ (10 mg/l)		Decanted	0.5	2.2	16		1437	
			Decanted	1	2.2	16		1469	

[a]Not determined

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INCIDENCE OF POTENTIALLY PATHOGENIC MICROORGANISMS IN FURTHER-PROCESSED TURKEY PRODUCTS[1]

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Abstract

Numbers of certain pathogenic microorganisms associated with turkey and turkey products were determined at three Minnesota turkey eviscerating and processing plants. The influence of processing and freezing was investigated. Using a predetermined sampling plan, skin and meat samples were obtained from 96 turkeys. Numbers of *Clostridium perfringens*, *Staphylococcus aureus*, salmonellae, and coliform organisms in each sample were determined. All types of organisms studied were found in 10 to 1000-fold higher levels in the skin than in meat samples. *Clostridium perfringens* and *S. aureus* organisms were recovered from samples obtained during each stage in the processing of the birds. Salmonellae were not found as frequently. Of 85 fresh skin and meat samples, 53 (62%) yielded *C. perfringens* and 46 (54%) yielded *S. aureus*. Salmonellae were recovered from 11 of 74 (15%) fresh samples and coliform organisms from 74 of 85 (85%) fresh samples. Frozen storage (31 days at −20 F) reduced recovery of the organisms to 56, 53, 9, and 67% respectively.

The increased production and consumption of turkey and further-processed turkey products, plus the continued reporting of food-borne illness attributed to turkey products, have created a need for more information on their microbiological quality. While there is information available on the occurrence of selected potentially pathogenic microorganisms associated with turkey products, there is relatively little information on the numbers of these organisms associated with turkeys processed under current commercial manufacturing conditions.

The first systematic study undertaken to determine the total numbers of selected microorganisms on turkey carcasses was that of Walker and Ayres *(19)*. They reported the presence and prevalence of coliform bacteria, staphylococci, fecal streptococci, and salmonellae on processed birds from five turkey processing plants. Other studies have dealt primarily with turkey products or selected organisms. Bryan et al. *(4, 5, 6)* investigated salmonellae associated with turkey products; Nivas et al. *(12)* identified salmonellae associated with various turkey processing plants in Minnesota; Salzer et al. *(15, 16, 17)* reported on salmonellae and other bacteria associated with turkey giblets. In these studies, the main emphasis was on detection of salmonellae, but some data were presented on numbers of mesophilic bacteria and the presence of other potentially pathogenic organisms such as *Staphylococcus aureus* or *Clostridium* spp. Recently, Mercuri et al. *(11)* published on the bacterial quality of precooked turkey rolls, and Zottola and Busta *(21)* reported on the microbiological quality of further-processed turkey products. Other related reports include the isolation of salmonellae and other air-borne microorganisms in turkey processing plants *(22)*, the microbiology of commercial turkey deboning *(3)*, the microbiological evaluation of mechanically deboned poultry meat *(14)*, distribution of clostridia in poultry processing plants *(10)*, and quantifying bacteria on poultry carcass skin and on subcutaneous bacteria in turkey carcasses *(1, 2)*. However, none of these reports contained quantitative or comprehensive information on the microbiological quality of turkeys being processed and further-processed in commercial operations.

The objectives of this study were to collect incidence data on numbers of *Clostridium perfringens*, *S. aureus*, salmonellae, and coliforms associated with turkey and turkey products produced under current manufacturing practices, and to evaluate the influence of processing and frozen storage on the numbers and incidence of these bacteria.

Materials and Methods

Sampling

Eight visits were made to three Minnesota turkey processing plants. Plant visitations were scheduled so that all samples from each visit could be obtained from a single flock during midday operations. Visits were at least one week but no more than two weeks apart. The plants visited were considered typical of commercial turkey evisceration and further-processing plants located in Minnesota.

Ninety-six turkeys were sampled, 12 during each plant visit. Sample birds were identified through use of predetermined sampling plans. At plants A and B, three birds about 100 birds apart on the eviscerating line were identified (1-3) before spin-chilling. In the same manner, three birds were identified (4-6) after spin-chilling and six birds were identified (7-12) at the end of the eviscerating line for sampling after overnight iced storage. Birds 10, 11, and 12 were sampled

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by plant personnel. At plant C, three sets of four birds each were identified about 100 birds apart on the eviscerating line. The first bird in each set was sampled before spin-chilling, the second bird after spin-chilling, and the third and fourth birds after overnight iced storage. The fourth bird in each set was sampled for breast and thigh meat by plant personnel; skin samples were taken experimentally.

Scald-tank and spin-chill tank water was sampled at the same time the sample birds were exposed to these steps in the processing. On four occasions, breast feathers were obtained from sample birds before they were scalded.

Skin samples

Skin samples were obtained by dissecting a flap of skin from the posterior ventral median sternum laterally approximately 7.5 cm on the right and left sides of the breast and anteriorly to include the neck skin. Skin samples collected from birds before spin-chilling, after spin-chilling, and after overnight iced storage were removed by using a sterile knife and sterile gloves for each bird to prevent carry-over of organisms from one bird to the next. The fourth skin sample was removed by personnel at plants A and B who used the same equipment (knives and gloves) as in their commercial deboning operations. The fourth set of skin samples from plant C was obtained experimentally using a sterile knife and gloves for each bird. All samples were placed in either commercially sterile polyethylene type bags or in sterile blender jars. The commercially removed skin samples from plants A and B and the fourth set of experimentally removed skin samples from plant C were frozen and held for 31 days before sampling. Frozen samples from plants A and B were held at −20 F in laboratory freezer space. Plant C frozen samples were held in plant freezer space at −30 F.

Breast samples

Breast samples were collected from birds after overnight iced storage. The breast of the third bird in each sample set from plant C and from birds 7, 8, and 9 at plants A and B was collected using the same knife and gloves as used in removing the skin sample. The breast of the fourth bird in each sample set from plant C and birds 10, 11, and 12 at plants A and B was commercially removed immediately after removal of the skin samples. Breasts from each bird were then placed into two sterile two-quart jars with one sample being retained for 31 days frozen storage.

Thigh samples

Thigh samples were collected in a manner similar to breast samples, except that single thighs were collected from two birds only on each plant visit. Samples were obtained from the first bird with the same knife and gloves used to collect the skin and breast samples from that bird. The second bird was sampled using commercial techniques by plant personnel. Samples were halved and placed in sterile jars for immediate culturing and for 31 days of frozen storage.

Water samples

Scald-tank and spin-chill water samples (200 ml) were collected in sterile jars at the times indicated above. Sodium thiosulfate was not added to the jars to neutralize residual chlorine. Identical water temperatures were being maintained at all three processing plants: scald-tank water, 144 ± 1 F; and chill-tank water, 59 ± 1 F.

Transport of samples

Samples were kept on ice and transported to the laboratory within 2 h of collection, except for frozen samples from plant C which were immediately placed in the plant freezer at −30 F and held for 31 days.

Deboning procedures

Experimental deboning was accomplished by using a sterile knife and gloves for each individual sample turkey. Samples were not permitted to touch other plant equipment such as conveyor belts and cutting boards. Likewise, commercial samples were taken from the fourth bird in each sample set of four birds, in the same manner except that the plant employees used their own routinely used knives and gloves. Therefore, carry-over of microbial contamination from one bird to the next was permitted with the commercially deboned birds by knives and gloves, but again, these samples were not permitted to touch other plant equipment.

These procedures should have permitted measurement of numbers of surface contaminants (*C. perfringens*, S. *aureus*, salmonellae, and coliforms) and of contaminants carried onto the meat samples by knives and gloves. They should have prevented, in the case of sanitarily deboned samples, carry-over from one bird to the next and in both instances minimized contamination by other environmental factors. Future studies will consider the contribution of other deboning equipment. The probability that the sample birds had systemic disease produced by *C. perfringens*, *S. aureus*, salmonellae, and coliform organisms was considered small; therefore, all of the organisms recovered from the meat samples were assumed to be due to surface (skin) sources.

Numbers of organisms

The numbers of organisms are reported as arithmetic ranges. Means and standard deviations were omitted, since variations in counts from one sample to another were relatively small with a small sampling. Whenever a $>$ (greater than) or $<$ (less than) value appears with one significant figure, e.g., <3, this indicates that all replicates were below the minimum for measurement.

The lower limits for the numbers of organisms (minimum quantity measurements) determined during this study were as follows: *C. perfringens* (MPN/g) <0.03, *C. perfringens* (CFU/g) <1.0, *S. aureus* (MPN/g) <3.0, salmonellae (MPN /g) <0.03, and coliforms (CFU/g) <1.0.

Laboratory procedures

Where applicable, laboratory procedures were identical to those previously reported by Zottola and Busta *(21)*. Numbers of *C. perfringens*, *S. aureus*, *Salmonella* spp., and coliforms were assessed. Portions of the sampled product (skin and meat) were weighed into sterile, tared, blender jars. A 1:10 dilution in sterile 0.1% peptone water was made and blended for 2 min in the case of skin and 1 min in the case of meat in an electric blender (Oster blender, operated at >3000 rpm.)

Clostridium perfringens

Enumeration and confirmation of *C. perfringens* was carried out using the procedure suggested by Hall *(9)*. Sulfite-Polymyxin-Sulfadiazine agar (SPS) incubated at 45 C was used. In addition, a triplicate tube dilution Most Pobable Number (MPN) determination and an enrichment technique both employing fluid thioglycollate were used. All enrichments were incubated at 45 C for 24 h. At the end of that time, a generous loopful was streaked onto SPS agar medium and the plates incubated anaerobically at 35 C for 24 h. The presence of *C. perfringens* was confirmed in the same manner as above. SPS agar medium was made from individual constituents immediately before use.

Staphylococcus aureus

The presence of S. *aureus* was determined as suggested by Olson *(13)* where Vogel Johnson medium (VJ) was used as

the plating medium and as suggested by Elliott *(7)* where Trypticase Soy Broth (TSB) with 6.5% NaCl was used in the triplicate tube per dilution Most Probable Number (MPN) determination. Data were reported when black colonies on VJ were observed and selected isolates proved to be coagulase-positive.

Salmonellae

Salmonella analysis was done as outlined by Galton et al. *(8)*. Triplicate tube, 10-fold dilutions (10, 1, and 0.1 g) were pre-enriched in lactose broth, enriched in tetrathionate broth, and streaked on Xylose Lysine Deoxycholate agar (XLD). Suspect colonies were tested biochemically in triple sugar iron, lysine iron agar, dulcitol broth, urea broth, and presumptive salmonellae were confirmed serologically with O-antigen and Spicer Edwards H-antigen antisera schemes. Serotypes were determined by the National Animal Disease Laboratory, Ames, Iowa.

Coliforms

Numbers of coliform organisms were determined by direct plating and Violet Red Bile agar (VRB) as suggested in Sharf *(18)*. Media were purchased from BBL or Difco.

RESULTS AND DISCUSSION

Clostridium perfringens

Table 1 presents a summary of data on the range of numbers (Colony Forming Units per gram) of *C. perfringens* found during the study and the fraction of total samples that yielded organisms for each category of sample. *C. perfringens* organisms were recovered from turkey skin and meat samples obtained at each stage tested. Numbers on feather samples ranged from 18 to 6500/g; numbers from scald water ranged from $<$ 1 to 3000/ml; numbers from spin-chill water were from $<$ 1 to 6/ml; a range of $<$ 1 to 10,000/g was observed on fresh skin samples; and in all instances $<$ 1 CFU/g of sample was recovered from frozen skin and from either fresh or frozen experimentally or commercially deboned turkey meat. Similar results were obtained using enrichment techniques and triplicate tube dilution Most Probable Number determinations. These data indicate that current processing techniques are able to rapidly reduce total numbers of *C. perfringens*, but also indicate that all turkey products may contain viable organisms. Even by experimentally deboning breast and thigh meat, thereby preventing carry-over of organisms from one bird to the next and contamination of the meat by processing equipment, 20 of 48 (40%) breast samples and 7 of 14 (50%) thigh samples yielded *C. perfringens* organisms. A similar occurrence of *C. perfringens* was reported by Mead and Impey *(10)* who studied poultry and turkey plants in England and found that clostridia could be readily isolated from carcasses at each stage tested in the processing of the birds.

It was evident in this Minnesota study that the numbers of *C. perfringens* present during initial pro-

TABLE 1. *Clostridium perfringens* IN ENVIRONMENT AND TURKEY OBTAINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (CFU/g - range)[b, c] Visit no.				Plant B (CFU/g - range)[b, c] Visit no.				Plant C (CFU/g - range)[b, c] Visit no.				Total
	1	2	3	Fraction[a]	1	2	Fraction[a]		1	2	3	Fraction[a]	
Feathers	6500	4500	*	2/2	18	20	*	*	*	*	*	*	4/ 4(100%)
Scald-tank water	(5.5-7.2)	(.8-2.3)	(43-55)	4/4	3000	(65-140)	9/9	(2-3)	(34-48)	(39-47)			19/19(100%)
Spin-chill water	<1	<1	<1	4/4	(3-6)	(1-3)	5/9	<1	<1	(.1-.3)			10/19(53%)
Skin:													
Before spin-chill	8/9 (1-<1)	(1-<1)	(1-<1)	6/6	(3500-10000)	<3000	7/9	<1	(<1-18)	(1-5)			21/24(87%)
After spin-chill	7/9 (2-<1)	(1-<1)	(.2-4.3)	6/6	(9-26)	<3000	7/9	<1	(<1-3)	(1-2)			20/24(83%)
After overnight storage in ice	7/9 <1	<1	(1.1-<1)	6/6	(5-99)	<1	6/9	<1	<1	<1			19/24(79%)
After 31 days frozen storage	6/9 <1	<1	<1	6/6	<1	<1	6/9	<1	<1	<1			18/24(75%)
Meat - after overnight storage in slush ice:													
Experimentally deboned breast	1/9 <1	<1	<1	6/6	<1	<1	4/9	<1	<1	<1			11/24(46%)
Commercially deboned breast	2/8 <1	<1	<1	6/6	<1	<1	7/9	<1	<1	<1			15/23(65%)
Experimentally deboned thigh	1/3 <1	<1	<1	2/2	<1	<1	1/2	*	<1	<1			4/ 7(57%)
Commercially deboned thigh	0/2 *	<1	<1	2/2	<1	<1	2/3	<1	<1	<1			4/ 7(57%)
Meat - after 31 days frozen storage:													
Experimentally deboned breast	3/9 <1	<1	<1	4/6	<1	<1	2/9	<1	<1	<1			9/24(38%)
Commercially deboned breast	2/8 <1	<1	<1	6/6	<1	<1	6/9	<1	<1	<1			14/23(61%)
Experimentally deboned thigh	1/3 <1	<1	<1	2/2	<1	<1	0/2	*	*	<1			3/ 7(43%)
Commercially deboned thigh	1/2 *	<1	<1	2/2	<1	<1	1/3	<1	<1	<1			4/ 7(57%)

*Not determined

[a]Number of samples positive/number of samples tested

[b]Colony Forming Units per gram (CFU/g)

[c]Minimum quantity measurement <1 organisms per gram of sample

TABLE 2. *Staphylococcus aureus* IN ENVIRONMENT AND TURKEY SAMPLES DETERMINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (MPN/g - range)[b, c] Visit no.				Plant B (MPN/g - range)[b, c] Visit no.			Plant C (MPN/g - range)[b, c] Visit no.			Total
	Fraction[a]	1	2	3	Fraction[a]	1	2	Fraction[a]	1	2	3	fraction[a]
Feathers	2/2	3.6	460	*	2/2	7.2	27	*	*	*	*	4/ 4 (100%)
Scald-tank water	2/5	3.6	<3	(<3-3.6)	3/4	(3.6-9.1)	(<3-23)	1/6	*	<3	(<3-3.6)	(%)
Spin-chill water	1/6	(<3-3.6)	<3	<3	2/4	(<3-3.6)	(<3-14)	1/9	(<3-9.1)	<3	<3	4/19 (22%)
Skin - breast:												
Before spin-chill	5/9	(3.6-43)	(<3-460)	(<3-3)	5/6	(<3-460)	(3.6-240)	7/9	(<3-23)	(15-21)	9.1	17/24 (71%)
After spin-chill	2/9	(<3-460)	(<3-3.6)	<3	6/6	(240-1100)	(7.3-23)	8/9	(9.1-23)	(24-93)	(<3-9.1)	16/24 (67%)
After overnight storage in ice	3/9	(<3-3.6)	(<3-3.6)	<3	6/6	(21-1100)	(23-1100)	8/9	(3.6-75)	(3.6-64)	(<3-43)	17/24 (71%)
After 31 days frozen storage	8/9	(7.3-39)	(23-43)	(9.1-43)	6/6	(43-150)	(23-150)	8/9	(<3-43)	(7.3-23)	(15-210)	22/24 (91%)
Meat - after overnight storage in slush ice:												
Experimentally deboned breast	0/9	<3	<3	3	4/6	(3.6-23)	(<3-36)	2/9	(<3-3.6)	3	(<3-3.6)	6/24 (25%)
Commercially deboned breast	1/8	<3	<3	(<3-91)	6/6	(9.1-93)	(14-43)	8/9	(<3-3.6)	(3.6-15)	3.6	15/23 (65%)
Experimentally deboned thigh	0/3	<3	<3	3	1/2	<3	3.6	1/2	*	9.1	<3	2/ 7 (28%)
Commercially deboned thigh	1/2	*	<3	3.6	2/2	1100	9.2	3/3	3.6	3.6	3.6	6/ 7 (87%)
Meat - after 31 days frozen storage:												
Experimentally deboned breast	3/9	<3	<3	(15-93)	4/6	(<3-9.1)	(<3-36)	3/9	<3	(<3-3.6)	(<3-9)	9/24 (38%)
Commercially deboned breast	2/8	<3	(23-93)	<3	4/6	(<3-9.1)	(<3-23)	4/9	3	(<3-9.1)	(<3-3.6)	10/23 (43%)
Experimentally deboned thigh	1/3	<3	<3	3.6	0/2	<3	3	0/2	*	<3	<3	1/ 7 (14%)
Commercially deboned thigh	1/2	*	<3	3.6	2/2	3.6	>1100	0/3	<3	<3	<3	3/ 7 (43%)

*Not determined
[a]Number of samples positive/number of samples tested
[b]Range per gram Most Probable Number (MPN/g)
[c]Minimum quantity measurement <3 organisms per gram of sample

cessing of turkeys varied from plant to plant. Data in Table 1 show that the number of organisms recovered from plant B sample birds, before and after spin-chilling, was from 100 to 1000 times greater than the number recovered from plants A and C sample birds. Week to week variations in numbers of organisms recovered were not obvious, indicating processing techniques may be directly responsible for the higher numbers of *C. perfringens* recovered from plant B birds.

Staphylococcus aureus

Estimates of the numbers of S. *aureus* organisms recovered from scald-tank water, spin-chill water, and turkey skin and meat samples are in Table 2. The fractions of total samples that yielded organisms for each category of sample are also presented. S. *aureus* organisms were recovered from skin and meat samples obtained at each stage tested. Numbers of 3.6 to 460/g were found on feather samples; numbers from scald water or chill water did not exceed 23/ml; numbers of < 3 to 1100/g were obtained from fresh skin samples; frozen skin samples yielded < 3 to 210/g; meat samples yielded < 3 to 1100/g.

These data imply that processing techniques, as used by these plants, did not materially reduce the numbers of S. *aureus* organisms present on turkey; however, a buildup of organisms did not occur during processing. Turkey meat samples, fresh or frozen, experimentally or commercially deboned, yielded numbers of organisms reflecting the numbers on their respective skin samples. There is evidence that carryover of organisms between birds and between skin and meat by deboning knives and gloves did take place. Recovery of S. *aureus* was made from 25 of 46 (54%) commercially deboned breast samples, while 15 of 48 (31%) experimentally deboned breast samples yielded the organism. Likewise, 9 of 14 (64%) commercially deboned thigh samples yielded organisms, while only 3 of 14 (21%) of the experimentally deboned thighs were found positive for S. *aureus* organisms.

This observed occurrence of S. *aureus* in the three Minnesota turkey processing plants is in sharp contrast to lack of this organism reportedly being recovered by Brant and Guion *(3)* in their survey of four commercial California turkey processing plants. They *(3)* reported that two of the California plants surveyed produced no samples that were positive for S. *aureus* organisms. However, they did indicate that their sample numbers were low and that a less inhibitory enrichment medium perhaps would have yielded more positive cultures. Other reasons for our more frequent recovery of S. *aureus* compared to the California study may include sampling and quantitating techniques. For example, in this study a whole skin blending technique was used, while Brant and Guion *(3)* used a swab sampling technique. As indicated by Avens and Miller *(1)*, swab techniques for sampling turkey products, especially skin, measure only the microorganisms on or under the loose outer surface skin cells. S. *aureus* isolated from feather follicles may pose just as serious a potential hazard as surface organisms.

Walker and Ayres *(19)* estimated the numbers of staphylococci associated with commercially produced turkeys during processing at < 10 to 3100/cm^2 on skin surfaces, and chill or scald water samples at < 500/ml. They estimated that 38% of their colonies were coagulase-positive. Two recent studies of further-processed turkey products have reported the presence of S. *aureus* organisms. Mercuri et al. *(11)* reported 8 of 28 (28%) foil-wrapped, baked, Eastern-type turkey rolls contained coagulase-positive staphylococci. Zottola and Busta *(21)* recovered coagulase-positive staphylococci from 24 of 35 (65%) raw further-processed turkey products. This emphasizes the necessity for adoption of standardized methods to isolate and enumerate S. *aureus* organisms in turkey products.

Salmonellae

Estimates of the numbers and incidence of salmonellae recovered during this study are in Table 3. Three (5%) isolations of salmonellae were made from 62 samples obtained during visits to plant A. One salmonellae-positive sample was a commercially removed skin sample frozen for 31 days at −20 F. Two were isolated from experimentally removed skin samples obtained from sample birds stored overnight in slush ice. No salmonellae were isolated from plant B samples. In contrast to this, 30 of 100 samples collected during three visits to plant C yielded salmonellae. Three (9.4%) of 32 samples were from the first visit to plant C. Two of these isolations were made from fresh commercially deboned breast meat. In the second visit to plant C, salmonellae were recovered from 14 (41%) of 34 samples and involved all 12 of the sample birds (100%). Isolations were made from: scald-tank water, 1 of 3 (33.3%); spin-chill water, 1 of 3 (33.3%); skin samples, 10 of 12 (83.8%); and from breast meat, 2 of 12 (16.6%). A similar wide distribution of salmonellae was found during the third visit to plant C where 13 (38%) of 34 samples and 9 (75%) of 12 sample birds yielded organisms. Isolations were accomplished from spin-chill water, 2 of 3 (66.6%); skin samples, 8 of 12 (66.6%); breast meat, 2 of 12 (16.6%); and from 1 of 4 (25%) thigh samples.

TABLE 3. *Salmonella* IN ENVIRONMENT AND TURKEY OBTAINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (MPN/g - range)[b, c] Visit no.			Plant B (MPN/g - range)[b, c] Visit no.			Plant C (MPN/g - range)[b, c] Visit no.			Total fraction[a]
	Fraction[a]	1	2	Fraction[a]	1	2	Fraction[a]	1	2	3	
Feathers	0/2	<.03	<.03	0/2	<.03	<.03	*	*	*	*	0/ 4 (0%)
Scald-tank water	0/4	<.03	<.03	0/4	<.03	<.03	1/9	<.03	(<.03-.11)	<.03	1/17 (6%)
Spin-chill water	0/4	<.03	<.03	0/4	<.03	<.03	3/9	<.03	<.03	(<.03-.36)	3/17 (18%)
Skin - breast:											
Before spin-chill	0/6	<.03	<.03	0/6	<.03	<.03	6/9	<.03	(.036-.93)	(.091-.21)	6/21 (28%)
After spin-chill	0/6	<.03	<.03	0/6	<.03	<.03	5/9	<.03	(.036-.15)	(<.03-.03)	5/21 (24%)
After overnight storage in ice	2/6	<.03	(<.03-.036)	0/6	<.03	<.03	5/9	<.03	(.091-.39)	(<.03-4.6)	7/21 (33%)
After 31 days frozen storage	1/6	<.03	<.03	0/6	<.03	<.03	2/9	<.03	(<.03-.15)	(<.03-.15)	3/21 (14%)
Meat - after overnight storage in slush ice:											
Experimentally deboned breast	0/6	<.03	<.03	0/6	<.03	<.03	1/9	<.03	<.03	<.03	1/21 (4.8%)
Commercially deboned breast	0/5	<.03	<.03	0/6	<.03	<.03	2/9	(<.03-.1)	<.03	<.03	2/20 (10%)
Experimentally deboned thigh	0/2	<.03	<.03	0/2	<.03	<.03	0/2	*	<.03	<.03	0/ 6 (0%)
Commercially deboned thigh	0/1	*	<.03	0/2	<.03	<.03	1/3	<.03	<.03	.07	1/ 6 (17%)
Meat - after 31 days frozen storage:											
Experimentally deboned breast	0/6	<.03	<.03	0/6	<.03	<.03	0/9	<.03	<.03	<.03	0/21 (0%)
Commercially deboned breast	0/5	<.03	<.03	0/6	<.03	<.03	4/9	(<.03-.036)	<.03	<.03	4/20 (20%)
Experimentally deboned thigh	0/2	<.03	<.03	0/2	<.03	<.03	0/2	*	<.03	<.03	0/ 6 (0%)
Commercially deboned thigh	0/1	*	<.03	0/2	<.03	<.03	0/3	<.03	<.03	<.03	0/ 6 (0%)

*Not determined
[a]Number of samples positive/number of samples tested
[b]Range per gram Most Probable Number (MPN/g)
[c]Minimum quantity measurement <.03 organisms per gram of sample

Fifteen salmonellae isolates from plant C were serotyped by the National Animal Disease Laboratory, Ames, Iowa. The serotypes identified from the first visit were *Salmonella newport* and *Salmonella saint-paul;* from the second visit, *Salmonella san-diego, Salmonella chester,* and six isolates of *Salmonella heidelberg;* and from the third visit, *S. chester* and four isolates of *S. heidelberg.*

These data indicate a possible contribution to the salmonellae "load" of turkey products by the deboning equipment (knives and gloves). Only 1 (2.4%) of 42 experimentally deboned breasts had recoverable salmonellae while organisms were recovered from (15%) of 40 commercially deboned breast samples. None of 12 experimentally deboned thigh samples yielded salmonellae while 1 (8.3%) of 12 commercially deboned thighs were found positive. Accompanying skin samples yielded salmonellae 10 (24%) of 42 times. Theoretically, contamination from the skin to the breast meat samples via knives or gloves would be the same in either experimentally deboned or commercially deboned samples, because the same knife and gloves were used to obtain experimentally deboned breast and thigh samples from an individual sample bird as was used to obtain the skin sample. Therefore, the increased recovery of organisms from the commercially deboned meat, compared to that of experimentally deboned meat, might be attributable to the buildup and/or transfer of salmonellae on the employees' knives and gloves. Ironically, this procedure of using the same knife and gloves to remove skin and meat samples from individual birds was not used in Plant C where all six of the salmonellae recoveries from commercially deboned breast meat were made. At Plant C, the breast skin was removed by the experimentalist from all sample birds using a sterile knife for each bird without excising the breast meat. Consequently, the salmonellae recoveries from these breast samples must be attributed to carry-over on the knives and/or gloves of the plant deboning personnel from previously deboned birds and not from the skin of the test bird. These findings are in agreement with the observations of Bryan et al. *(5)* who reported an increase in isolations of salmonellae from finished products compared to carcasses before further processing.

Coliforms

Enumeration of coliform organisms was included in this study, based on their role as sanitation indicator organisms in the food industry, although their use in the poultry processing industries has not been extensive. According to Zottola and Busta *(21),* use of coliforms as a sanitation index in further-processed turkey products has limitations because these organisms are part of the natural flora indigenous to turkeys. Wilkerson et al. *(20)* indicated that coliforms were of equal value to enterococci in indicating initial contamination. However, coliforms failed to survive freezing and, therefore, lacked value as indicators on frozen turkey products. It is possible that coliforms could be used as indicator organisms for cooked further-processed turkey products where isolation of these bacteria should be an indication of contamination after cooking. Brant and Guion *(3)* reported that the results of their study of California turkey processing plants confirm that coliforms are not a good index of sanitation. They found that sanitation measures which appear to reduce total counts also appear to encourage coliforms. Walker and Ayres *(19)* in 1959 noted that if coliforms occur in unusually great numbers, undue contamination from fecal material and filth and the lack of careful handling during processing would be indicated.

Coliform organisms were recovered from all of the turkey carcasses sampled and from 204 (80%), of 257 various samples, 4 (100%) of 4 feather samples, 3 (19%) of 16 scald-tank water samples, 18 (95%) of 19 spin-chill water samples, and from 23 (82%) of 28 thigh meat samples (see Table 4).

Enumeration procedures indicated that coliforms were present at each stage tested in the processing of the birds. The greatest numbers of coliforms were recovered from skin samples before and after spin-chilling. Subsequent skin samples, after the birds had been stored overnight in slush ice, generally showed a reduction in numbers of organisms. These samples yielded from 4 to 550 organisms per gram, excluding samples from visit one to plant B where undue contamination was evident in numbers ranging from 570 to 4400/g. These coliform counts appear higher than those reported by Walker and Ayres *(19)* who found coliform numbers on the skin surface of turkeys to range from 10 to 70/cm^2 with the usual range from 10 to 30. The significance of these observations may have been reduced because Walker and Ayres utilized cotton swab sampling techniques over 2 cm^2 areas delineated by metal guides, while our results were obtained by determining the number of organisms in 200 to 500 g of blended skin tissue. According to Avens and Miller *(1),* the cotton swab method for quantifying bacterial populations on turkey carcass skin enumerated only 22% of the bacteria measured by the skin tissue blending method. Considering this difference in recovery, the coliform counts obtained in our study were even nearer to the values of Walker and Ayres *(19)* and to those of Brant and Guion *(3)* where they found the mean coliform counts from various plants ranged from 0 to 1010/cm^2.

Results of this study substantiate the limitations on

TABLE 4. COLIFORMS IN ENVIRONMENT AND TURKEY OBTAINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (CFU/g - range)[b, c] Visit no.			Plant B (CFU/g - range)[b, c] Visit no.			Plant C (CFU/g - range)[b, c] Visit no.			Total
	Fraction[a]	1	2	3	Fraction[a]	1	2	Fraction[a]	1	2	3	fraction[a]
Feathers	2/2	680	440	*	2/2	10000	2300	*	*	*	*	4/ 4 (100%)
Scald-tank water	0/6	<1	<1	<1	1/4?	<1	(<1-70)	2/6	*	(<.1-1.6)	(<.1-.2)	3/16 (19%)
Spin-chill water	5/6	(<1-6)	(13-64)	(11-39)	4/4	(1000-1300)	(1700-3100)	9/9	(11-20)	(31-43)	(71-93)	18/19 (95%)
Skin - breast:												
Before spin-chill	9/9	(9-24)	(26-170)	(4-220)	6/6	(5600-28000)	(90-280)	9/9	(310-470)	(290-2800)	(490-640)	24/24 (100%)
After spin-chill	9/9	(9-45)	(100-120)	(55-690)	6/6	(1500-16000)	(100-250)	9/9	(100-370)	(270-480)	(350-560)	24/24 (100%)
After overnight storage in ice	9/9	(4-19)	(14-290)	(12-76)	6/6	(570-4400)	(45-100)	9/9	(100-550)	(360-530)	(110-210)	24/24 (100%)
After 31 days frozen storage	6/9	(<1-27)	(2-7)	(14-310)	6/6	(140-680)	(24-52)	7/9	(<1-1)	(3-6)	(15-43)	19/24 (80%)
Meat - after overnight storage in slush ice:												
Experimentally deboned breast	3/9	(<1-1)	(<1-1)	(<1-3)	5/6	(9-30)	(<1-8)	9/9	(2-26)	(5-17)	(3-5)	17/24 (71%)
Commercially deboned breast	6/8	(<1-7)	(6-25)	(<1-14)	6/6	(26-930)	(5-28)	9/9	(4-70)	(14-72)	(6-15)	21/23 (95%)
Experimentally deboned thigh	1/3	1	<1	<1	2/2	18	13	2/2	*	1	8	5/ 7 (70%)
Commercially deboned thigh	2/2	*	11	2	2/2	40	47	3/3	4	47	17	7/ 7 (100%)
Meat - after 31 days frozen storage:												
Experimentally deboned breast	0/9	<1	<1	<1	5/6	(2-8)	(<1-2)	5/9	(<1-1)	(<1-1)	(2-3)	10/24 (41%)
Commercially deboned breast	5/8	(<1-1)	(1-5)	(<1-28)	6/6	(55-70)	(3-27)	6/9	(1-5)	<1	(1-3)	17/23 (73%)
Experimentally deboned thigh	1/3	<1	<1	1	2/2	13	4	2/2	*	1	4	5/ 7 (70%)
Commercially deboned thigh	2/2	*	3	6	2/2	47	25	2/3	<1	6	4	6/ 7 (86%)

*Not determined
[a]Number of samples positive/number of samples tested
[b]Colony Forming Units per gram (CFU/g)
[c]Minimum quantity measurement <1 organism per gram of sample

TABLE 5. FREQUENCY OF ISOLATION OF SELECTED MICROORGANISMS FROM FRESH AND FROZEN TURKEY SKIN, BREAST, AND THIGH MEAT

Sample	*C. perfringens**	*S. aureus**	Salmonellae*	Coliforms*
Skin:				
Before spin-chilling	21/24 (87%)	17/24 (71%)	6/21 (28%)	24/24 (100%)
After spin-chilling	20/24 (83%)	16/24 (67%)	5/21 (24%)	24/24 (100%)
After overnight storage of whole birds in slush ice	19/24 (79%)	17/24 (71%)	7/21 (33%)	24/24 (100%)
After 31 days frozen storage	18/24 (75%)	22/24 (91%)	3/21 (14%)	19/24 (80%)
Breast meat:				
After overnight storage of whole birds in slush ice	26/47 (55%)	21/47 (45%)	3/41 (7%)	38/47 (80%)
After 31 days frozen storage	23/47 (50%)	19/47 (40%)	4/41 (10%)	27/47 (57%)
Thigh meat:				
After overnight storage of whole birds in slush ice	8/14 (55%)	8/14 (55%)	1/12 (8%)	12/14 (86%)
After 31 days frozen storage	7/14 (50%)	4/14 (29%)	0/12 (0%)	11/14 (79%)

*Number of positive samples/number of samples tested.

the use of coliforms as indicators of potentially pathogenic organisms. Recoveries of salmonellae were successfully accomplished from four of nine Plant C commercially deboned, frozen breast meat samples, yet coliform numbers were found to be only <1 to 5/g. Likewise, no salmonellae were recovered from Plant B commercially deboned, frozen breast meat samples, yet the number of coliforms were estimated from 3 to 70/g. Thus, the lack of apparent coliforms cannot guarantee a freedom from potentially pathogenic organisms, nor can the presence of coliforms necessarily indicate the presence of potentially pathogenic organisms. Obviously, the numbers of coliforms may be used to indicate relative sanitation and processing conditions in the plant.

The levels of all types of microorganisms studied were greater in skin samples than in equivalent meat samples. This differential ranged from 10-fold to 1000-fold greater populations from skin compared to the meat. Frozen skin samples yielded potential pathogens from 14 to 91% of the time (Table 5): *C. perfringens*, 18 (75%) of 24; *S. aureus*, 22 (91%) of 24; salmonellae, 3 (14%) of 21; and coliforms, 19 (80%) of 24. The implications of these findings may be obvious, yet the solution to the problem is not readily evident. One cannot discard skin from further-processed turkey products without adversely affecting flavor and profits any more than one can market skinless turkeys. All types of microorganisms studied were recoverable from fresh and frozen meat samples at a sufficiently high level that even if skin was not included in further-processed turkey products, essentially all raw further-processed turkey products would contain some potentially pathogenic microorganisms.

The numbers of organisms recovered from different sources varied from plant to plant. Plant B samples yielded from 10- to 100-fold more microorganisms than did samples from Plants A or C; however, no salmonellae were recovered from Plant B samples. Plant A samples yielded the fewest numbers of organisms. Plant C appeared to have been processing salmonellae infected birds. During the second visit, 12 (100%) of 12 and during the third visit, 9 (75%) of 12 carcasses sampled yielded salmonellae. During the first visit to plant C, salmonellae were recovered from three breast meat samples, yet no skin sample recoveries were accomplished, indicating that either infected birds were being processed or a build-up of organisms from previously processed infected birds had occurred. Recovery levels from breast and thigh meat obtained by the experimentalist, when compared to levels with similar commercially deboned meat, indicate that there probably was a contribution by processing personnel to the microbial "load" of the samples. Coliform bacteria isolated from the various plants tended to be associated with higher numbers of *C. perfringens* and *S. aureus;* however, no obvious correlation between numbers of salmonellae and coliform bacteria can be made from this study. The effect of freezing on the frequency of recovery and on the apparent numbers of these unwanted microorganisms does not appear significant. Although, after 31 days of frozen storage there is a trend towards reduced incidence of recoverable organisms, especially with *C. perfringens* and coliforms, the incidence rates were still considerable. Consequently, product freezing and storage at currently available commercial freezer temperatures will provide no assurance of complete destruction of all potentially pathogenic organisms.

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SOURCES OF SALMONELLAE CONTAMINATION OF MEAT FOLLOWING APPROVED LIVESTOCK SLAUGHTERING PROCEDURES. II

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ABSTRACT

Samples were taken from 218 animals of 3 species slaughtered at 3 plants to determine the spread of bacterial contamination during slaughter. Salmonellae and *Escherichia coli* were cultured from swabs taken of the equipment during slaughter, from various carcass sites, and from fecal samples. The study indicated that some equipment contamination occurred during slaughter and that carcass washing did not remove contaminants but simply washed them lower on the carcass. Rumen/cecum samples were most effective for isolation of salmonellae from the gastrointestinal tract. The average level of salmonellae contamination of the carcass for all species was 10% and of the processed product, 2%. There were no salmonellae isolated from cattle carcasses. Isolation of the bung (rectum) with a plastic bag did not reduce contamination but sterilization of the bung dropper's knife between carcasses reduced the incidence by an average of greater than 50%. Salmonellae were isolated from boneless mutton but not from raw or cooked pork and beef products. Isolations from the hide were closely related with carcass contamination. Enrichment and non-enrichment media isolations of salmonellae were closely related.

In spite of the large amount of work that has been done to reduce its incidence, salmonellosis remains as one of the major food-borne hazards to human health. In a review of the literature, Newell and Williams *(10)* reported that excretion of salmonellae by livestock increased from the farm to the slaughtering plant. They also reported various factors in slaughtering plant design, such as air movement and carcass washing, which affect salmonellae contamination of the carcass. Pether and Gilbert *(11)* demonstrated that *Salmonella anatum* survived for 3 h on the fingers of food plant workers and that organisms could be isolated from workers' hands following a 15-sec handwash with warm water. Minimal inoculation of workers' hands was sufficient to contaminate meat products. *Escherichia coli* was also isolated from the fingers of 13 out of 110 butchers.

Knivett *(7)* reported that 25% of the carcasses of chickens subclinically infected with *S. typhimurum* and subsequently treated with furazolidone were found to be contaminated following processing. Chlorination of the carcass chill water at the level of 200-250 ppm significantly reduced the number of contaminated carcasses. Bicknell *(3)* reported on *S. aberdeen* infection in a cattle herd grazing land irrigated with sewer effluent. There were no reported human cases of salmonellosis from the area of the effluent.

Matches and Liston *(8)* reported that salt concentrations sufficient to prevent growth of salmonellae at low temperatures might not be sufficient to prevent growth at higher temperatures. Also, they found that salmonellae could grow only over a narrow pH range at low temperatures *(9)*. In two articles Ray et al. *(13, 14)* indicated that samples of non-liquid products taken at the beginning of a day's production were more likely to give accurate data on salmonellae contamination that those taken later. In a companion paper *(14)* there authors reported that extended storage may reduce salmonellae contamination but will not eliminate it completely.

Enkiri and Alford *(5)* reported that low-incidence strains of salmonellae were more susceptible to dry and frozen storage while higher-incidence serotypes showed longer survival in frozen storage. Stersky et al. *(15)* reported that survival of air-borne *S. newbrunswick* was quite variable in a food processing plant and that long-term survival could present a definite public health hazard. Baldwin et al. *(2)* presented evidence to show that microwave heating of some fish products did not kill all salmonellae. Three hundred ninety seconds were required to obtain a lethal dose in some fish products, a period which exceeds the normal heat time in microwave ovens.

Bailey et al. *(1)* traced an outbreak of *S. panama* enteritis to infected ham from a plant in which one worker was excreting the organism. Sewer samples from the plant also yielded the organism. After removal of the infected worker the organism could no longer be isolated from the sewage. A later human outbreak was traced to infected hams that had been served in the plant canteen. Examination of swine subsequently slaughtered at the plant was negative. Price et al. *(12)* reported that pooling of pre-enrichment lactose broth culture was effective in screening multiple blood samples.

TABLE 1. SALMONELLAE AND *E. coli* ISOLATIONS FROM 74 CATTLE

Locations	Control (33)		Bung bagged (25)		Bung knife sterilized (16)	
	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)
Body cavities	0	62	0	49	0	29
Midline	0	73	0	44	0	19
Vertebra and neck	0	42	0	40	0	38

TABLE 2. SALMONELLAE AND *E. coli* ISOLATIONS FROM 64 SWINE

Locations	Control (34)		Bung bagged (10)		Bung knife sterilized (20)	
	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)
Body cavities	17	49	7	70	2	38
Midline	24	62	0	40	15	20
Vertebra and neck	19	51	10	60	5	25

TABLE 3. SALMONELLAE AND *E. coli* ISOLATIONS FROM 40 SHEEP

Locations	Control (30)		Bung knife sterilized (10)	
	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)
Body cavities	5	70	0	50
Midline	17	42	0	50
Neck	0	69	0	0

TABLE 4. SALMONELLAE AND *E. coli* ISOLATIONS FROM 19 CATTLE AND 21 SWINE BEFORE AND AFTER WASHING

Locations	Before washing		After washing	
	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)
Pelvic cavity	2	33	2	43
Abdominal cavity	5	48	0	53
Midline	5	63	5	45
Thoracic cavity	2	45	2	48
Vertebra	2	40	5	40
Neck	2	50	2	45

This research was the continuation of a previous, more limited study *(4)*.

EXPERIMENTAL METHODS

Microbiological samples from cattle, swine, and sheep were taken at 3 meat packing plants on 1 day each month for 19 months. Approximately 12 animals of the same species were sampled each month. Animals to be sampled the following day were randomly selected from the holding pens and identified. A sterile swab was user to take samples from the rectum at the time of arrival at the plant and immediately before slaughter. The animal's temperature was taken on arrival and before slaughter. Five 10-g samples of holding pen droppings were taken on the day of slaughter.

Before the beginning of slaughter a 100-ml sample of hog-scald-tank water was taken. A 100-inch^2 area of two viscera pans and the head wash cabinet was swabbed midway through the day's operations using a sterile template to describe the area. Two 100-ml samples of water from the sterilizers were taken during the day's operations and the temperature recorded. With two sterile swabs an area of approximately 100-inch^2 on each carcass was swabbed from the midline, the abdominal cavity, the thoracic cavity, the pelvic cavity, sawed vertebral surfaces, and the neck. During the last one-third of the study the ventral surface of the hide was also swabbed before opening. Ten-gram samples of rumen contents from cattle and sheep and swabs of cecal contents of swine were taken. Swine cecal contents were too watery to permit collection of solid samples. All samples were immediately chilled and held at 3 C until cultures were initiated.

Carcass identity was maintained throughout the processing operation. After further processing, consumer-size packages of products manufactured with meat from the sampled carcasses were taken. Both raw and cooked products were sampled. Since the sheep slaughtering plant produced only boneless manufacturing mutton, samples of boneless mutton were taken. All product samples were frozen at −18 C until analyzed.

The samples of fecal material were divided and handled in the same manner as the two swabs. Difco media were used in this study. The two swabs and two fecal material samples were used to initiate cultures in lactose broth and tetrathionate broth, both of which were incubated for 24 h at 35 C. After incubation the tetrathionate broth was used to inoculate brilliant green sulfadiazine (BGS) and salmonella - shigella (SS) agars which were incubated for 24 h at 35 C; and the lactose broth was used to inoculate tetrathionate broth and eosin-methylene blue (EMB) agar. The EMB agar was incubated for 24 h at 35 C. Presumptive *E. coli* colonies were confirmed using IMVIC reactions. Nine typical salmonellae colonies per plate were inoculated into triple sugar iron and lysine iron agar and incubated for 24 h at 35 C. Those showing typical salmonellae reactions were confirmed by somatic and flagellar antigens. Processed meat samples were handled in the same manner using a 30-g sample according to the methods described by Galton et al. *(6)*.

RESULTS AND DISCUSSION

During the 19-month sampling period 93 cattle, 85 swine, and 40 sheep were sampled. The number of sheep sampled is low because the cooperating plant closed and there were no other sheep slaughtering facilities within a reasonable distance.

For the first 6 months samples were collected from animals slaughtered using routine procedures to establish a base-line of salmonellae and *E. coli* incidence. These incidence rates are shown in Tables 1-3 in the "Control" column. Following this, the role of carcass washing in spreading contamination was investigated (Table 4). It appears that washing has little effect on the level of carcass contamination. From these data, one must question the practice of permitting large amounts of fecal material to be washed off of the carcass instead of being trimmed.

Two modifications of slaughtering practices to reduce carcass contamination were evaluated. One was bagging the bung (rectum) at the time of dropping. A large, non-sterile plastic bag was placed over the bung and tied in place before dropping it into the abdominal cavity. As seen in Tables 1 and 2 bung bagging had some effect on reducing car-

cass contamination in cattle but in swine it decreased the level of salmonellae contamination. Apparently the pelvic cavity became contaminated from the worker's hands and knife during bung dropping with the outside of the plastic bag being contaminated in the pelvic cavity and contaminating other parts of the carcass. This technique was not used on sheep since the bung is not routinely tied during evisceration.

The second modification was to sanitize the butcher's knife immediately before dropping the bung. These results are shown in Tables 1-3. This technique was successful in reducing both salmonellae and *E. coli* contamination in all species. In the case of sheep, the results may not be accurate since the sheep bung-dropper understood very little English and probably did not sterilize his knife as intended.

TABLE 5. SALMONELLAE AND *E. coli* ISOLATIONS FROM EQUIPMENT IN SWINE, CATTLE, AND SHEEP SLAUGHTER PLANTS

	Swine - 8 times		Cattle - 7 times		Sheep - 4 times	
Locations	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)
Sterilizers (174-180)	0	0	0	0	0	0
Viscera pans	13	38	0	29	50	75
Head cabinet	—	—	14	43	—	—
Scald tank (137-144)	13	0	—	—	—	—

TABLE 6. SALMONELLAE ISOLATIONS FROM INTESTIONAL AND FECAL SAMPLES OF SWINE, CATTLE AND SHEEP

Location	Swine (%)	Cattle (%)	Sheep (%)
Rectal after arrival	13	0	20
Rectal before slaughter	22	1.5	20
Rumen or cream	32	3	38
Pen droppings	23	9	10

TABLE 7. SALMONELLAE ANL *E. coli* ISOLATIONS FROM SAMPLES OF RAW AND COOKED PROCESSED PRODUCTS

	Raw		Cooked	
Products and number of samples	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)
Beef				
Bonless - 13	0	0	—	—
Hamburger - 11	0	0	—	—
Pork				
Chops - 13	0	0	—	—
Sausage - 27	0	0	0	0
Mutton				
Bonless - 20	10	0	—	—

TABLE 8. SALMONELLA AND *E. coli* ISOLATIONS FROM HIDE AND CARCASS OF CATTLE AND SWINE

	Swine (23)		Cattle (19)	
Location	Sal. (%)	*E. coli* (%)	Sal. (%)	*E. coli* (%)
Hide	13	37	0	57
Carcass	22	57	0	63

Equipment isolations are shown in Table 5. It does not appear that equipment sanitizing was adequate in all cases. Perhaps 180 F water should be used on both the visera pan and head wash cabinet between each carcass instead of only when they have been obviously contaminated. Though the sterilizer temperatures were sometimes below 180 F no isolations were made from the water. Salmonellae was isolated from the hog scald tank water one time.

Table 6 shows the isolations of salmonellae from the rectum after arrival and before slaughter, from holding pen droppings, and from the rumen or cecum, depending upon the species. As expected, the number of isolations increased between the time of arrival and the time of slaughter *(10)*. However, rumen/cecum isolations were highest of all, perhaps indicating that this might be the best location for sampling. Results from the processed meat samples are in Table 7. *E. coli* was not isolated from the processed products and salmonellae were isolated only from 2 boneless mutton samples.

E. coli contamination of the carcass was high in all species, averaging 59% in cattle, 54% in swine, and 60% in sheep. Salmonellae isolations were zero from cattle, 20% from swine, and 7% from sheep. Some of the sheep slaughtered during the study were in poor condition and stress by drought and may have been excreting organisms in excess of what would normally be expected.

To monitor the hide's role in carcass contamination, sterile swabs were made of the midline area before opening the hide or skin (swine) for evisceration. The skin of 23 swine was sampled after removal from the dehairer and 19 cattle were sampled before opening the midline. No sampling was done on the hide of sheep. These results are in Table 8. A total of 100 isolations of salmonellae were made. Of this number 55 were made on both enrichment (lactose and tetrathionate) and non-enrichment (tetrathionate only) media. Twenty nine isolations were made on non-enrichment media only and 16 were made on enrichment media only.

The average level of salmonellae contamination of the carcass was 10% and of the processed products, 2%. This study does not show salmonellae to be a problem in cattle but that a problem does exist in swine. The salmonellae isolations from sheep must be evaluated carefully in view of the unusual climatic conditions existing at the time of the study. Sterilization of the bung dropper's knife reduced salmonel-

lae contamination from 10% to 2.5% and *E. coli* contamination from 58% to 30%.

The following bioserotypes of *Salmonella* were detected: *S. java*, 21.15%; *S. derby*, 24.04%; *S. anatum*, 15.38%; *S. newlands*, 10.58%; *S. heidelberg*, 7.00%; *S. oranienberg*, 6.00%; and miscellaneous and those not assignable to types, 15.85%.

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H. L. Thomasson, Managing Editor

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FOR SECOND VICE-PRESIDENT AND SECRETARY-TREASURER

DAVID DEAN FRY

Dave Fry, presently, assistant to the Plant manager, at T. G. Lee Foods, Orlando, Florida has been with the company nineteen years as laboratory technician, producer relations and fieldman, plant purchasing, and supervisor of quality control.

Dave was born in Hanna, Alberta, Canada, July 25, 1928. He obtained his elementary and high school education in Indiana, Illinois, Arizona and California and a Dairy Science degree from Purdue University. He served six and one half years at Purdue Creamery in the position of milk procurement, quality control and plant management.

He was a member of the U. S. Army Paratroops, 82nd Airborne Division, and is a member of Reeves Memorial United Methodist Church, Masonic Lodge #293, Orlando, Florida, Independent Order of Foresters, 19 years FAMFES – 8 years on Board of Directors – 2 years as Chairman of the Laboratory Section and as President in 1969. He was Finance Chairman of Local Arrangements Committee for IAMFES annual meeting in 1967, Florida Affiliate Membership Chairman 1972 and is Chairman of Local Arrangements Committee for the forthcoming annual meeting in St. Petersburg, Florida.

Dave has been very active in industry and civic affairs, Treasurer and President of T. G. Lee Employees Credit Union, President of the Central Florida Credit Union Chapter 1960, member of Florida Credit Union League Board of Directors 1963, Pesticide Committee of Florida Dairy Products Association, Chairman of Butter Fat Committee for Florida Milk Commission and member of Florida Dairy Quality Milk Council, Boy Scout Leader for 2 years – Merit Badge Counselor 3 years, presently Dairying and Public Health Merit Badge Counselor for area.

Dave has also continued his education, completing additional College Credit courses in, marketing, supervision management problems, accounting and computer science. His hobbies are citizens band radio and gardening. He is married 26 years to Iris E. Fry, they have three children: Virginia age 24, married and living in Portland, Oregon; Donald age 21, Jeanne age 8 in Orlando and their home is 704 Terrace Blvd.

———————

DONALD J. PUSCH

Don Pusch is Manager, Technical Quality Assurance with The Pillsbury Company, Minneapolis, where his basic responsibility is in the area of microbiology as it relates to the company's food safety program.

A native Minnesotan, Don served in the U. S.

Navy from 1944 to 1946. He received a B.A. in Bacteriology in 1949 and an M.S. in Food Science & Industries in 1970 from the University of Minnesota.

Upon his graduation in 1949, he joined the Minnesota Department of Agriculture as a bacteriologist. During his tenure there, he advanced to the position of Senior Microbiologist in charge of the Microbiology laboratory Survey in the Division of Laboratory Services. He was also the Milk Laboratory Survey Officer. Over the past several years, Don has been involved in the presentation of the University of Minnesota Extension Short Courses in the area of food sanitation and milk and food laboratory practices. He joined The Pillsbury Company in 1972.

Don has been active in a number of state and international organizations where he has served in the following capacities: Past President and currently on Executive Board, Minnesota Sanitarians Association; Past President, Henrici Society for Microbiologists; Executive Committee Member, Minnesota Section, I.F.T.; Member of Subcommittee on Laboratory Methods for the Examination of Milk and Milk Products and Subcommittee for Examination of Foods, International Ass'n. of Milk Food and Environmental Sanitarians. In addition, he is a member of Gamma Sigma Delta Honor Society of Agriculture, National I.F.T., American Society for Microbiology, The Society for Applied Bacteriology and A.O.A.C.

Don lives with his wife and three children in St. Paul, Minnesota.

SECRETARY-TREASURER

RICHARD PELL MARCH

Richard P. March is a professor in the Department of Food Science at the New York State College of Agriculture, Cornell University, Ithaca, New York. Until 1965, he devoted 75% of his time to extension work as a specialist in milk quality and fluid milk handling and processing, and the balance of his time in research and teaching courses in fluid milk processing and quality control. At present, extension accounts for 95% of his time with 5% for research activities.

He was raised in Massachusetts, majoring in dairy industry at the University of Massachusetts, receiving a B.S. degree in 1944. After a tour in the U. S. Marine Corps, he entered the Graduate School at Cornell University to major in dairy industry, receiving an M. S. degree in 1948.

Professor March taught a one-year program in dairy manufacturing until its termination in 1951, at which time he was promoted from instructor to assistant professor. He became an associate professor in 1955, and full professor in 1965. In 1965 he also became department extension leader and is still serving in this capacity.

He is active in the New York State Association of Milk and Food Sanitarians, serving as secretary-treasurer from 1957 and executive secretary since 1967, secretary of the Dairy Industry Equipment Committee from 1952-57, secretary of the Farm Practices Committee from 1955-62, and secretary of the Council of Affiliates since 1952. He is a member of the International Association of Milk, Food, and Environmental Sanitarians, serving as Secretary-Treasurer since 1970, a member of their Farm Methods Committee from 1959-65, secretary of their Council of Affiliates in 1961, and chairman in 1962 and 1963.

In both the State and International Associations he has served as chairman of a number of subcommittees including the Uniform Milkhouse Plans for the Northeast, Milk Transfer Systems, Sediment Testing, and Training Programs for Bulk Tank Truck Operators, and co-chairman of the Northeast Committee on Uniform Guildlines for Loose Housing Systems. In 1963 he was the recipient of the New York State Association's Dr. Paul B. Brooks Memorial Award for outstanding contributions to the organization; in 1972 he received the Emmet R. Gauhn Memorial Award for outstanding service to the State Association.

Professor March has been chairman of the Northeast Dairy Practices Council, an 11 state organization, since its inception in 1969.

COLUMBUS, OHIO PUBLIC HEALTH OFFICIAL GIVEN NATIONAL VENDING INDUSTRY AWARD

Ray B. Watts, chief of the sanitation division of the Ohio Department of Health, Columbus, O., was honored with the highest award of the National Automatic Merchandising Association (N A M A) for major contributions to the field of sanitation and safety of vending machines.

Watts, who was instrumental in establishing the first statewide vending inspection program in the United States in 1959, was presented with the Arthur J. Nolan Award, given only four times since it was first awarded in 1966.

Watts has represented the American Public Health Association on the Automatic Merchandising Health-Industry Council (AMHIC) since 1958 and has contributed in significant ways to the advancement of the vending industry's self-regulation program, according to N A M A officials.

A graduate of William and Mary College and the University of Michigan, Watts has been engaged in environmental health work for 33 years.

Cooperation of the vending industry with state and local health officials through AMHIC led to a model federal sanitation code for vending machines in 1957 and widespread adoption of standard inspection and training of health inspectors and vending company personnel, N A M A officials said. The program was started in the early 1950's and Watts has been one of the chief architects of the program.

The award was presented at the annual meeting of N A M A during the opening session of the association's 27th annual meeting. More than 10,000 persons were registered at the convention and trade show which took place from October 18 through 21.

LAND O'LAKES MILK MANAGER RETIRES

Farris Biggart, manager of the Land O'Lakes Grade A Fluid Milk Division at Cedar Rapids, Iowa, retired November 2, after 28 years in the dairy industry.

He is 61 years of age and was employed by Sanitary Farm Dairies in Cedar Rapids in 1961. When Land O'Lakes purchased the dairy in 1969, he was Farm Department Field manager.

In his most recent position, Biggart was responsible for receiving, dispatching and other managerial duties connected with the handling of Grade A milk purchased by Land O'Lakes from producers in the Cedar Rapids-Clinton area.

NEW ACTION COMMITTEE NATIONAL RESTAURANT ASSOCIATION

L. Eugene Johnson, Jr. of Louisville, Ky., has been elected national chairman of the Action Committee of the National Restaurant Association, a slect leadership group created to advance the programs and purposes of the NRA.

At the first annual meeting of the 50-state Action Committee, held May 20, during the NRA 54th Annual Restaurant, Hotel-Motel Show in Chicago, NRA President Richard Harman heralded the formation of the group as "a very important step forward for the Association."

Purpose of the Action Committee is to increase individual membership involvement and participation in the activities of the NRA. Among its goals are:

1. To alert, acquaint and attune local NRA members and all interested parties to those NRA activities deemed worthy of food-service operator support.

2. To provide the NRA with a "grass roots" vehicle whereby local information could be quickly gathered, and positive action taken, regarding present problems, opportunities and trends.

3. To help provide meaningful direction for the future NRA activities and programs.

MARTIN NAMED EDITOR OF CULTURED DAIRY PRODUCTS JOURNAL

Effective January 1, 1974, Dr. James H. Martin, Professor and Head, Dairy Science Department, South Dakota State University, Brookings, South Dakota 57006, will become the Editor of the Cultured Dairy Products Journal. Dr. Martin replaces Dr. H. C. Olson, recently retired from Oklahoma State University, as the Journal Editor.

EXTENSION AWARD RECEIVED BY JAMES W. CROWLEY

The distinctive requirements of an effective extension worker are combined in the documentary evidence supporting this year's recipient of the DeLaval Extension Award. Repeatedly, co-workers, superiors, and clientele attest to his ability to communicate effectively, translate research into workable programs, and motivate with the professional skill and attitude of an accomplished teacher.

Dr. James W. Crowley's academic career was interrupted by service in the U. S. Navy during World War II where he attained the rank of lieutenant. He earned the Ph.D. degree in dairy nutrition and biochemistry in 1951 at the University of Wisconsin. While major efforts in his 23-year extension career have been in the nutritional area, Crowley has exhibited unusual versatility by spearheading programs in dairy records, AI technician training, off-campus graduate level training management, and development of a state dairy cattle grading program and herd health clinics.

In his major field of endeavor, dairy cattle nutrition, he has enabled the dairymen of Wisconsin to increase their efficiency of production with maximum use of home-grown rations. For nine years he has contributed a feeding column twice each month to a nationwide dairy publication.

Crowley's campus activities include membership in the Faculty Senate and five university extension committees, training of college and 4-H judging teams, advisor to Alpha Gamma Rho, and active participation in the Saddle and Sirloin and Badger Dairy Clubs. He has participated in seminars and conferences in Canada, England, Brazil, and numerous states.

In 1969 he received a University Extension Excellence in Teaching Award. In 1970 he was granted the USDA Superior Service Award.

Kentucky born in 1921, he received his B.S. and M.S. degrees at his home state university. While there, he was chancellor of Alpha Zeta, president of the Agricultural Council, and held numerous other offices. He was selected as the Outstanding Senior in the University of Kentucky. He is a member of Sigma Xi, Gamma Alpha, Omicron Delta Kappa, and for 25 years a member of ADSA. Crowley has participated in programs at the IAMFES Annual Meeting and at the Wisconsin Association of Milk and Food Sanitarians Annual Meeting.

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JOURNAL OF MILK AND FOOD TECHNOLOGY

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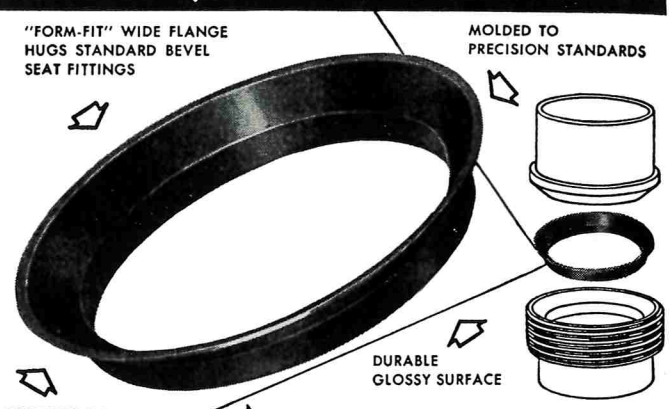

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Dairy authorities speak out on better cow milking

William L. Arledge
Director of Quality Control
Dairymen, Inc.

Quality milk makes consumers happy and helps dairymen prosper.

Dairy farmers are unique. In most cases their primary source of income is their milk check. A major influence on that milk check is quality, and it has been shown that, as the quality of milk in a given market improves, so does consumer demand, and so does income to the dairy farmer.

High quality milk starts with the cow but it must be continued each step of the way until the milk is purchased and consumed.

TASTE MAKES FRIENDS

The nutritional value of milk to the human diet is never disputed. The relative dollar value of these nutrients, particularly protein in relation to other food costs, is also not disputed. However, consumers could care less about protein and nutritional value if the milk does not taste good due to poor quality control practices somewhere between the cow and the consumer. People simply will not buy poor quality milk.

Over 90% of consumer complaints are the result of post-pasteurization contamination and improper temperature control of the bottled product after pasteurization. This is dramatized by the fact that seven Grade A dairy plants recently increased the shelf life of their total packaged products from an average of 6 days to more than 21 days by improving in-plant controls over post-pasteurization contamination. Little or no change was made in the quality of the raw milk coming into the plant. (This shows that significant improvements can be made in dairy plants as well as on farms.)

REVIEW YOUR EFFORTS

Your role of producing quality milk daily can be as simple as you desire to make it.

It has been proven many times that it is cheaper to spend whatever is necessary to thoroughly clean your bulk tank, pipeline, or bucket milkers (all milk contact surfaces) than it is to do an improper job.

Follow these recommendations:

1. Read the label of your chlorinated cleaner detergent and *measure* the correct amount of water to be added to the correct amount of cleaner.
2. Be sure when circulating cleaning systems to start your wash cycle with 160° F. water and *stop* circulating when the temperature drops to 110° F. (If you don't, you will redeposit soil and fat.)
3. In colder areas, the use of a heating element in the wash tank is recommended to help maintain wash temperatures above 110° F., preferably 130-140° F. (Six to ten minutes is usually enough*).
4. Many dairy barns do not have hot water heaters that will deliver water at 160-180° F. and even if they do—the recovery of temperature in the heater is too slow and you end up trying to wash in lukewarm to cool water.
5. To prevent loss of *hot* water for cleanup, many dairymen install a second hot water heater and set the regulator at around 110-120° F. for use in hand washing of cows' teats and udders prior to milking as well as other manual cleaning chores. This leaves your other hot water heater available for the important job of cleaning equipment.
6. If you have an electric hot water heater, install fast recovery heating elements to prevent cool down of your hot water. For safety make sure all heaters are equipped with an approved *pressure* and *temperature* relief valve.
7. After thorough washing of equipment, rinse in an acid rinse and then immediately prior to milking always *sanitize* all milk contact surfaces with an approved sanitizer.

CARELESSNESS IS A LUXURY

As surprising as it may seem, we still find some people rinsing equipment with plain water after sanitizing. This causes great problems affecting milk quality since all water supplies contain from a few to large numbers of the "cold loving" psychrothrophic bacteria that can and do cause some very undesirable off-flavors in milk. Granted these organisms are killed by pasteurization but, by carelessness, dairymen can alter the flavor of milk prior to its leaving the farm by 1) lack of sanitizing, or 2) rinsing equipment rather than washing, or by 3) only rinsing a bulk tank.

Too many times we see dairymen doing a good job in their milking management practices, but disregarding their water supply, temperature of cleaning solutions and sanitization, thus nullifying all other good practices.

Quality of your *only* product affects *your only* income; your milk check. You can do more concerning the quality of your milk! Follow the routine and procedures you know to be correct and with these few quality tips, you may prevent the shipment of a tank of less-than-superior-quality milk.

We must all relate to the consumer. Do a quality job in your personal operation and expect the same throughout the chain of events to the consumer. You will reap the benefits through personal pride "all the way to the bank".

*Refer to local Health Department regulations

This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.